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Fast gradient screening of pharmaceuticals with 5 cm long, narrow bore reversed-phase columns packed with sub-3 μm core–shell and sub-2 μm totally porous particles

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The performance of 5 cm long narrow-bore columns packed with 2.6–2.7 μm core–shell particles and a column packed with 1.7 μm totally porous particles was compared in very fast gradient separations of polar neutral active pharmaceutical compounds. Peak capacities as a function of flow-rate and gradient time were measured. Peak capacities around 160–170 could be achieved within 25 min with these 5 cm long columns. The highest peak capacity was obtained with the Kinetex column however it was found that as the flow-rate increases, the peak capacity of the new Poroshell-120 column is getting closer to that obtained with the Kinetex column. Considering the column permeability, peak capacity per unit time and per unit pressure was also calculated. In this comparison the advantage of sub-3 μm core–shell particles is more significant compared to sub-2 μm totally porous particles. Moreover it was found that the very similar sized ($d_p = 2.7$ μm) and structured ($\rho = 0.63$) new Poroshell-120 and the earlier introduced Ascentis Express particles showed different efficiency. Results obtained showed that the 5 cm long narrow bore columns packed with sub-3 μm core–shell particles offer the chance of very fast and efficient gradient separations, thus these columns can be applied for fast screening measurements of routine pharmaceutical analysis such as cleaning validation.

1. Introduction

High separation efficiency and faster analysis have always been of great interest in liquid chromatography. A new period has been started with using sub-2 μm fully porous particles, silica monolithic rods and shell particles [1–4].

On sub-2 μm particles, due to the narrow peaks, sensitivity and separation are improved at the cost of pressure. Ultra-high pressure pump systems (UHPLC or VHPLC) have been used to overcome the high pressure drop generated by small particles of packing materials [1,2]. It was demonstrated that the analysis time could be reduced to a few minute interval without the loss of resolution and sensitivity using very fine porous particles [3,4]. The commercialization of UHPLC or VHPLC systems has accelerated applications of fast liquid chromatography in pharmaceutical analysis [5–10].

Temperature in liquid chromatography also offers a possibility to cut down the analysis time. Elevated temperature reduces the viscosity of mobile phase and hence increases the mass transfer. The separation time can be shortened significantly without loss of resolution through column and mobile phase heating [11–14]. However, this strategy suffers of limitations such as the small number of stable packing materials at temperatures higher than 80 °C as well as the potential degradation of thermolabile analytes and the need to have a constant temperature along the chromatographic system. Therefore, until now, the pharmaceutical industry has not considered this approach routinely [15].

Monolithic columns were introduced for their potential use at high mobile phase velocities due to decreased mass transfer effects over conventional fully porous particles [16,17]. The monolith approach, originally initiated by the work of Hjertén et al. [18], Svec et al. [19], Horvath and co-workers [20], Tanaka and co-workers [21], which already lead to a number of well performing, commercially available polymeric and silica monolith columns [22,23]. Due to the low phase and surface area per column ratio, retention on monolithic columns is generally lower compared to packed columns.

The development of core–shell or “superficially porous” silica particles was considered as a breakthrough in column technology aimed at reducing analysis times while maintaining column efficiencies and requiring relatively low back pressures [24,25]. A major benefit of the core–shell particles is the small diffusion path (0.23–0.50 μm) compared to fully porous particles (e.g.,

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1.7–1.9 μm). In theory, decreasing the thickness of the porous layer of porous material should cause a decrease of the C term in the van Deemter plot, because the length along which molecules should diffuse decreases [26]. Besides their structure, made of a shell around a solid core particle, the interesting feature of these superficially porous particles is their extremely narrow particle size distribution (PSD). Different authors report that this narrow PSD is the key for their success at separating small molecules [27–29]. Now core–shell packing materials are commercially available in different particle diameters (1.7 μm, 2.6 μm, 2.7 μm and 5 μm) with different shell thickness (0.23 μm, 0.25 μm, 0.35 μm and 0.50 μm).

The aim of this study was to make a practical evaluation of the possibilities of commercially available columns packed with sub-3 μm core–shell particles in gradient elution mode. We measured and compared the peak capacities for a mixture of 14 polar neutral active pharmaceutical ingredients (API, low molecular weight steroid and non-steroid hormone compounds, MW: 270–430 g/mol) which are often separated in pharmaceutical routine analysis. Three core–shell packing of similar size (Poroshell 120 SB-C18, Kinetex C-18 and Ascentis Express C-18) and a column packed with totally porous sub-2 μm particles (Waters Acquity BEH C18) were applied in order to compare the efficiency of the short, narrow bore reversed-phase columns in fast gradient elution. The peak capacities were measured with the same samples on each column, at constant chromatographic linear velocity and gradient steepness. We focused on fast gradient separations (gradient time was varied between 3 and 18 min) what can be useful for screening purposes for the measurements of hormones, steroids and their degradants, thus short (5 cm long) narrow bore (2.1 mm internal diameter) columns were used in this work. The fast screening gradient separations are very useful in pharmaceutical cleaning control analysis and in impurity testing of APIs.

2. Experimental

2.1. Chemicals, columns

Acetonitrile (gradient grade) was purchased from Merck (Darmstadt, Germany). For measurements water was prepared freshly using Milli-Q® equipment (Milli-Q gradient A10 by Millipore).

The test analytes were polar neutral small pharmaceutical active ingredient (API) compounds. Their molecular weights are in the range of 270–430 g/mol. All 14 test compounds (steroids, non-steroid hormones such as estrogens, progestins and anti-androgens and their main degradation products such as: gestodene (13-ethyl-17-hydroxy-18,19-dinor-17α-pregna-4,15-dien-20-yn-3-one), levonorgestrel (13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one, (−)), estradiol (Estra-1,3,5(10)-triene-3,17β-diol), dienogest (17α-cyanomethyl-17β-hydroxyestra-4,9(10)-diene-3-one), finasteride (N-tert-butyl-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide), noretistherone-acetate (17-acetoxy-19-nor-17α-pregn-4-en-20-yn-3-one), bicalutamide (N-[4-cyano-3-(trifluoromethyl)phenyl]-3-(4-fluorophenyl)sulfonyl)-2-hydroxy-2-methyl propanamide, (±)), tibolone (17-hydroxy-7α-methyl-19-nor-17α-pregn-5(10)-en-20-yn-3-one), ethinyl-estradiol (19-nor-17-pregn-1,3,5(10)-trien-20-yn-3,17-diol) and 5 degradation products (impurities) of ethinyl-estradiol (different hydroxy-, keto- and dehydro-ethinyl-estradiol degradants) were produced by Gedeon Richter Plc (Budapest, Hungary).

The Kinetex core–shell C18 column packed with 2.6 μm shell particles (50 mm × 2.1 mm) were obtained from GEN-Lab Ltd, Budapest. Ascentis Express C18 column (Supelco) with a particle size of 2.7 μm (50 mm × 2.1 mm) was purchased from Sigma–Aldrich Ltd., Budapest. Waters UPLC™ BEH C18 column with a particle size of 1.7 μm (50 mm × 2.1 mm) was purchased from Waters Ltd., Budapest. The recently introduced Poroshell 120 SB-C18 column with a particle size of 2.7 μm (50 mm × 2.1 mm) was a generous gift from Kromat Ltd., Budapest. All of the columns used in this study were new no other experiments were performed on them.

2.2. Equipment, software

All measurements were performed using a Waters Acquity system equipped with binary solvent delivery pump, an auto sampler and a photo diode array detector (Waters Ltd. Budapest, Hungary). The UPLC system had a 5 μl injection loop and a 500 nl flow cell (path length = 10 mm). A polyether ether ketone (PEEK) tube (15 cm × 0.1 mm) is located between the column outlet and the detector. The overall extra-column volume (V_{ext}) is 12 μl as measured from the injection seat of the auto-sampler to the detector cell at 1 ml/min. The measured dwell volume is 130 μl. Data acquisition with an 80 Hz data sampling rate and instrument control were performed by Empower 2 Software (Waters).

The non-linear curve fitting to peak capacity plots was performed using Statistica 9.0. Image J (freeware image-processing software program developed at the National Institutes of Health) was used to determine the particle size and the size distribution of column packing materials.

2.3. Apparatus and methodology

The mobile phase “A” was pure water while the mobile phase “B” was pure acetonitrile. The eluents were degassed by sonication for 5 min.

The stock solutions of each test solute were set in acetonitrile (1000 μg/ml). The stock solutions were sonicated for 5 min in ultrasonic bath then they were homogenized. After that 1 ml stock solutions of each solute were transferred into a 50 ml volumetric flask. A sample-solvent of acetonitrile/water 40/60 v/v was added to the flask to give the final volume. The concentration of the final pharmaceutical test mix was 20 μg/ml (for each solute).

Preliminary gradient runs were performed to find the final gradient experimental conditions. According to the preliminary results, a linear gradient from 40% B to 90% B provides sufficient retention on all four columns and baseline resolution at least for 8–9 compounds.

The volume fractions of acetonitrile at the beginning and at the end of the gradient were set constant at 40 and 90%, respectively ($\Delta\varphi = 0.50$). It was followed by column equilibration (at the end of each gradient run, the 90% B was held for 1 min then the initial gradient condition was reset and run for 2 min to equilibrate the system). Solvent strength was varied linearly with times ranging from 3 to 21 min ($t_g = 3, 6, 9, 12, 15, 18$ and 21 min) being employed. The efficiency of each column was investigated at six different chromatographic linear velocities ($u_0 = 0.144, 0.193, 0.241, 0.289, 0.337$ and 0.385 cm/s). The columns were thermo-stated at 30 °C. The injected volume was 0.5 μl (partial loop with needle overfill mode), and UV detection at 215 nm (80 Hz) was applied. Since all experimental parameters have been kept constant, these conditions can be used to effectively compare the efficiency of the 5 cm long 2.1 mm internal diameter core–shell columns in fast gradient separation. For each linear velocity, six different gradient times t_g were applied in order to allow a direct comparison of the peak capacity P_c between the four columns at constant gradient analysis time. A total of $6 \times 7 \times 4 = 168$ chromatograms were recorded, corresponding to six different linear velocities, seven different gradient time and four different columns.

The extra-column variance of our UPLC system was measured around 6–7 μl^2 depending on the flow-rate [41,42]. In gradient elution mode the samples concentrate at the inlet of the column, therefore only the contribution of the connecting tube after the column and the detector cell contribute to the peak broadening. This extra-column peak variance in gradient elution mode was found to be negligible.

3. Results and discussion

3.1. Efficiency in gradient elution mode

There are a number of objective measures of chromatographic performance, for example, peak efficiency, resolution, and peak capacity. Of these, resolution and peak capacity are relevant to gradient systems. Peak capacity, a concept first described by Giddings [30] and soon put to good use by Horváth for gradient chromatography [31]. It is a measure of the separation power that includes the entire chromatographic space together with the variability of the peak width over the chromatogram. For samples, which contain many components, peak capacity is a useful measure of the comparative separating power of different columns. In gradient separation, peak capacity is a function of column efficiency, gradient time, flow rate, and analyte characteristics.

The general expression of the theoretical peak capacity P_c in liquid chromatography, assuming a resolution of unity between the successively eluted peaks can be written as [32]:

$$P_c = 1 + \int_{t_I}^{t_F} \frac{1}{4\sigma} dt \tag{1}$$

where t_I is the retention time of the first eluted peak, t_F is the retention time of the last eluted peak, dt is a dummy time variable, and σ is the time standard deviation of a peak.

For the practical comparison of different columns' efficiency in gradient elution mode, two experimental formulas are often used. The conditional peak capacity is directly related to the average peak resolution [33] and is computed from experimental data as

$$n_c = \frac{t_{R,n} - t_{R,1}}{w} \tag{2}$$

where $t_{R,n}$ and $t_{R,1}$ are the retention times of the last and the first eluting peaks and w is the average 4σ peak width. Here n_c is called as conditional peak capacity, because it depends strongly on all of the experimental conditions of the gradient elution program including temperature, flow-rate, initial and final mobile phase compositions, as well as the column parameters and the sample's properties [34].

If the peak width pattern over a chromatogram is similar, as it is in most reversed-phase gradient separations, one can use the following popular formula of experimental peak capacity [35–37]:

$$P_c = 1 + \frac{t_g}{\bar{w}} \tag{3}$$

where w is the average baseline peak width, and t_g is the gradient run time. The procedure of Eq. (3) is also applicable for implementation in generic methods, where the gradient run time is a predetermined value [38].

By analogy to Knox's separation impedance concept [39], similar representation of efficiency can be constructed by calculating the peak capacity per unit time and per unit pressure values, according to the next formula [40]:

$$PTP = \frac{P_c}{t_g \cdot \Delta p} \tag{4}$$

where PTP is the peak capacity per unit time and per unit pressure value, t_g is the gradient run time and Δp is the column pressure drop.

Table 1
Some physicochemical data of the four packing. Stationary phase, particle size (d_p), particle size distribution ($d_{90/10}$), ratio of the diameter of the solid core to that of the particle (ρ), average pore size (D_{pore}).

	Stationary phase	d_p [μm]	$d_{90/10}$	ρ	D_{pore} [nm]
Waters BEH	C18	1.81	1.53	1.00	13.9
Kinetex	C18	2.50	1.15	0.73	9.5
Ascentis Express	C18	2.72	1.16	0.63	9.3
Poroshell-120	C18	2.80	1.11	0.63	11.9

3.2. Physical properties of the column packing

At first for a basic comparison some physical properties – which can have major effect on the column efficiency – were measured.

Scanning electron microscopic (SEM) images and Image-J (image-processing software) were used to determine the particle size distribution of the Kinetex, Ascentis Express and Waters BEH particles. For the calculation of particle size distribution 300 individual particles were considered. The diameters of the particles were measured along the horizontal direction. The average particle diameter value and the particle size distribution of the new Poroshell packing was taken from literature [43]. The mean diameters and the particle size distribution ($d_{90/10}$) are summarized in Table 1. SEM pictures of the Kinetex, Waters BEH and Ascentis Express particles were already presented in our previous studies [41,42].

Low-temperature nitrogen adsorption (LTNA) measurements were performed to determine the average pore-size (D_{pore}) of the particles. Nitrogen adsorption/desorption isotherms were measured at 77 K, using a Quantachrome Nova 2000E computer controlled apparatus. Samples were outgassed at 120 °C for 24 h. The average pore size estimated from the Brunauer–Emmett–Teller (BET) isotherm parameters were obtained as 9.5 nm for Kinetex 2.6 μm packing, 9.3 nm for Ascentis Express particles and 13.9 nm for Waters BEH 1.7 μm material. The pore size of the new Poroshell packing was measured by the manufacturer (D_{pore} = 11.9 nm).

Generally the benefit of core–shell particles is explained with the short diffusion path, and the significance of the ratio (ρ) of the diameter of the solid core to that of the particle is always emphasized as a measure of column efficiency. Beside this ratio (ρ) many other physical properties such as particle diameter, particle size distribution and pore diameter affect the column efficiency. This is why we measured and collected the main physical properties of the packing (Table 1).

3.3. Peak widths, chromatograms

A key consideration in estimating peak capacity from experimental data is the choice of the peaks to be used for peak capacity calculation. Fig. 1 shows the chromatograms generated using 5 cm long narrow bore columns with a gradient time of 3 min, at the flow rate of 0.8 ml/min, while Fig. 2 displays another case with a relative long gradient time (18 min), and decreased flow rate (0.4 ml/min). It can be obviously seen that the selectivity of the four C18 columns is significantly different and some of the peaks cannot be separated with baseline resolution, moreover the selectivity depends also on the gradient steepness. The Poroshell-120 material shows significantly different selectivity for our real-life test compounds than it is in the case of the other three studied C18 phases.

The experimental determination of peak capacity was based on the measurement of the baseline peak widths of nine compounds dispersed across the gradient window and having similar peak height (see Figs. 1 and 2). These chosen compounds eluted with higher resolution than $Rs > 1.5$ on each four column thus the measurement of peak width at baseline were practicable. Eq. (3) was used to calculate the peak capacity values. Peak capacity of

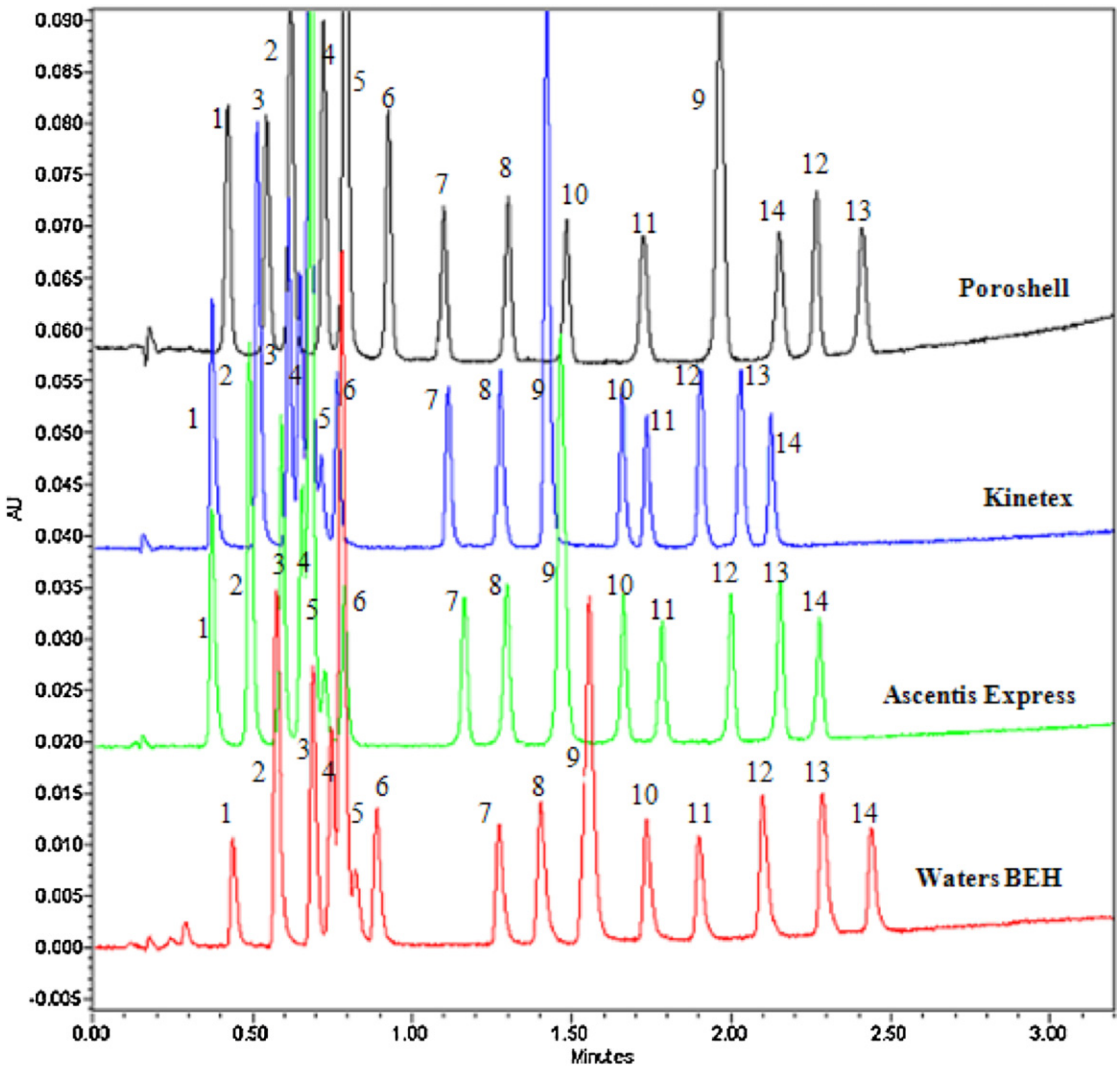

Fig. 1. Representative chromatogram of test samples. Conditions: the volume fractions of acetonitrile at the beginning and at the end of the gradient were set at 40 and 90%, the columns (5 cm × 2.1 mm) were thermo-stated at 30 °C, the injected volume was 0.5 μl. The gradient time was set as 3 min, at the flow-rate of 0.8 ml/min. Analytes: degradation products/impurities of ethinyl-estradiol (1,2,4,5,13), dienogest (3), ethinyl-estradiol (6), estradiol (7), finasteride (8), bicalutamide (9), gestodene (10), levonorgestrel (11), tibolone (12), and noretisterone-acetate (14). Peak 1,6,7,8,9,10,11,12,13 and 14 were considered for peak capacity calculations.

all four columns was calculated at each gradient span and linear velocity.

3.4. Peak capacity

In this study, the influence of the mobile phase linear velocity and of the steepness of gradient on the column performance was investigated under gradient elution conditions. These two experimental variables are directly related to the mobile phase flow-rate and the gradient time duration which are often used to adjust a proper separation in practical every day work. The former has a direct influence on the column efficiency while gradient duration plays an important role on the resolution under gradient conditions. The peak capacity of 5 cm long narrow bore Kinetex C18, Poroshell 120 SB C18, Ascentis Express C18 and Waters BEH C18 columns were calculated and plotted against flow-rate and gradient time according to Eq. (3) (Fig. 3). These results illustrate how considerably the experimental conditions affect the peak capacity of similar sized core–shell packings and a fully porous one as well as demonstrate some differences in column efficiency. It should be stressed that these conditions are not meant to maximize the peak capacity for each column as higher peak capacity can be achieved by choosing longer gradient times. Instead, we merely wanted to identify a set of conditions that allows a fair comparison within a practically acceptable time frame, especially for short narrow bore columns.

Shell particles have a thick porous layer, thus it is expected that the axial and eddy dispersion contributions to the efficiency of columns packed with these particles would correspond to the external diameter of the particle, but the internal mass-transfer resistances would correspond to the thickness of the porous layer. According to the theory the efficiency of core–shell columns depends on the ratio (ρ) of the diameter of the solid core to that of the particle in a core–shell particle [26]. As this ratio increases the mass transfer kinetics becomes faster through the shell particles than it is through totally porous particles. This ratio is $\rho = 0.73$ for Kinetex and $\rho = 0.63$ both for Ascentis Express and Poroshell. The nominal diameters of the particles are very similar ($d_p = 2.6$ μm for Kinetex and $d_p = 2.7$ for Ascentis Express and Poroshell) thus similar efficiency can be expected for Ascentis Express and Poroshell while the Kinetex column probably provides higher separation power. At first sight (Fig. 3) the performance achieved with 2.6–2.7 μm core–shell particles are comparable to those obtained with finer fully porous particles (1.7 μm) in case of

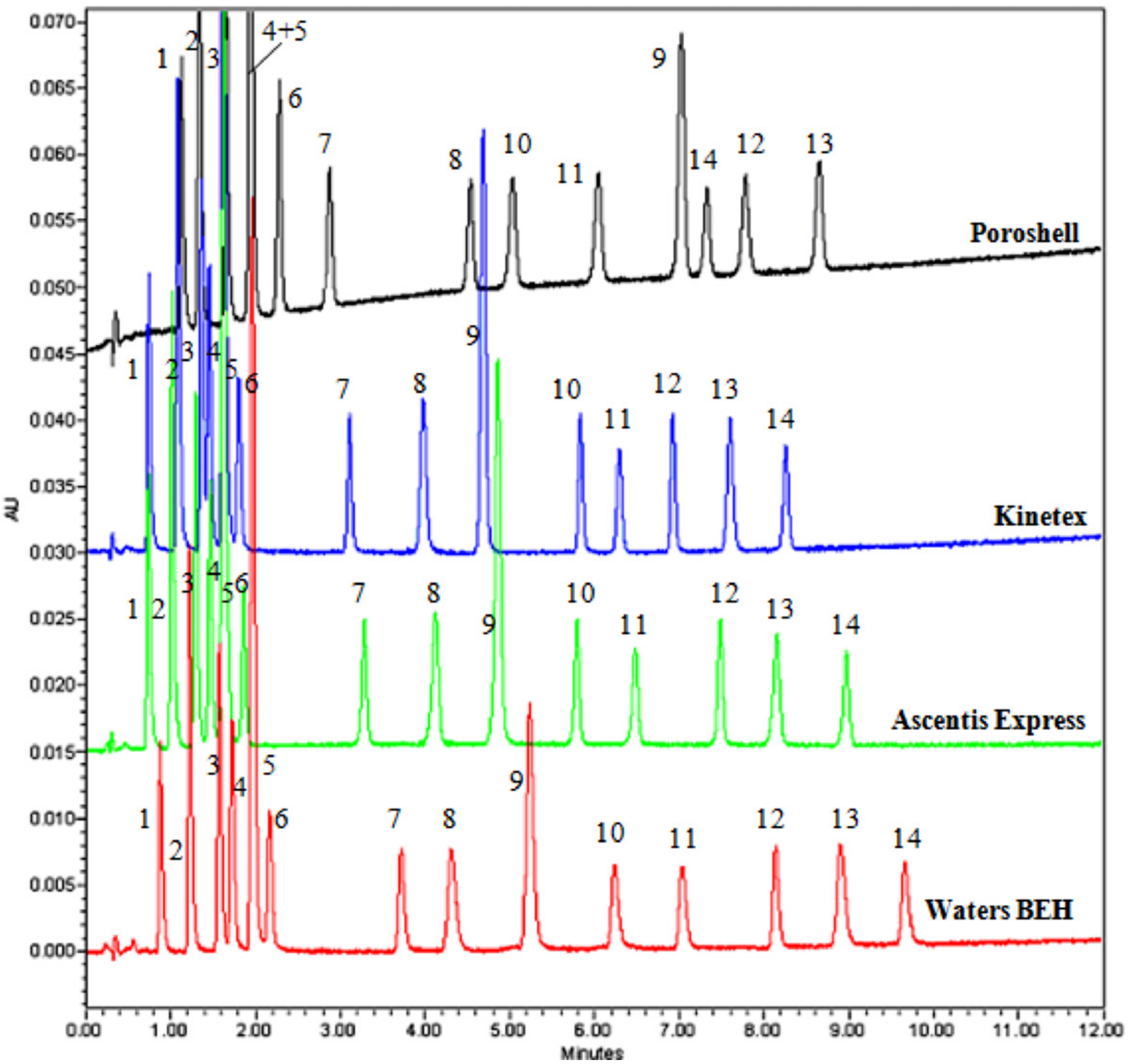

Fig. 2. Representative chromatogram of test samples. Conditions: the volume fractions of acetonitrile at the beginning and at the end of the gradient were set at 40 and 90%, the columns (5 cm × 2.1 mm) were thermo-stated at 30 °C, the injected volume was 0.5 μl. The gradient time was set as 18 min, at the flow-rate of 0.4 ml/min. Analytes: degradation products/impurities of ethinyl-estradiol (1,2,4,5,13), dienogest (3), ethinyl-estradiol (6), estradiol (7), finasteride (8), bicalutamide (9), gestodene (10), levonorgestrel (11), tibolone (12), and noretistherone-acetate (14). Peak 1,6,7,8,9,10,11,12,13 and 14 were considered for peak capacity calculations.

fast gradient separations and the results are in agreement with the theory.

When very fast gradient separation was achieved (3 min gradient time), the Kinetex column outperformed all the other three tested columns at all applied flow-rates (Fig. 4A). At 0.3 ml/min flow-rate the Kinetex column performed a peak capacity value of $P_c = 35$, while the Ascentis Express, Poroshell and Waters BEH column achieved a $P_c \sim 28$. In the range of 0.3–0.5 ml/min flow-rate, the Ascentis Express, Poroshell and Waters BEH column provided practically the same efficiency. If the flow-rate was higher than 0.5 ml/min the column packed with fully porous sub-2 μm particles performed lower peak capacity than those obtained with core–shell particles. At high flow-rate (0.8 ml/min) the Kinetex column yielded an efficiency of $P_c = 55$. The Ascentis Express, Poroshell and Waters BEH gave about 10%, 20% and 25% lower peak capacity than Kinetex when 0.8 ml/min flow-rate was applied. Results showed that in the case of a 3 min gradient span, the Ascentis Express column gradually outperforms the Poroshell column when the flow-rate is increased ($F > 0.5$ ml/min) in spite of the same shell thickness and particle diameter. Another finding is that the slope of the peak capacity curve of Waters BEH column is flatter than it is for the columns packed with larger partially porous particles. A possible explanation of this phenomenon could be due to the heat friction released at high flow rates. The heat power friction released in the BEH column per volume unit is typically twice larger than that released in the columns packed with 2.6–2.7 μm particles [44].

When a relatively slow gradient separation was executed (flat gradient program with a gradient time of 21 min), significantly different results were obtained than it was in the case of very fast (steep) gradient separation (Fig. 4B). The first interesting result is that the core–shell type Ascentis Express packing produced lower peak capacity than the Waters BEH column which is packed with totally porous particles. Probably it can be explained by the rough surface of Ascentis Express particles in which the mass transfer rate is reduced through the outer stagnant liquid [45,46]. The other surprising result is that as the flow-rate increases, the peak capacity of the Poroshell column ($\rho = 0.63$) is getting closer to that obtained with the Kinetex column ($\rho = 0.73$). The Poroshell column provides 15% less peak capacity at 0.3 ml/min, while at enhanced flow-rate (0.8 ml/min) it performs only 2% less peak capacity than it is in the case of Kinetex. A probable reason of this result can be the larger average pore size of Poroshell material which could have some benefit when longer separations are performed. The average pore size of the new Poroshell material is 11.9 nm while in the case of Kinetex

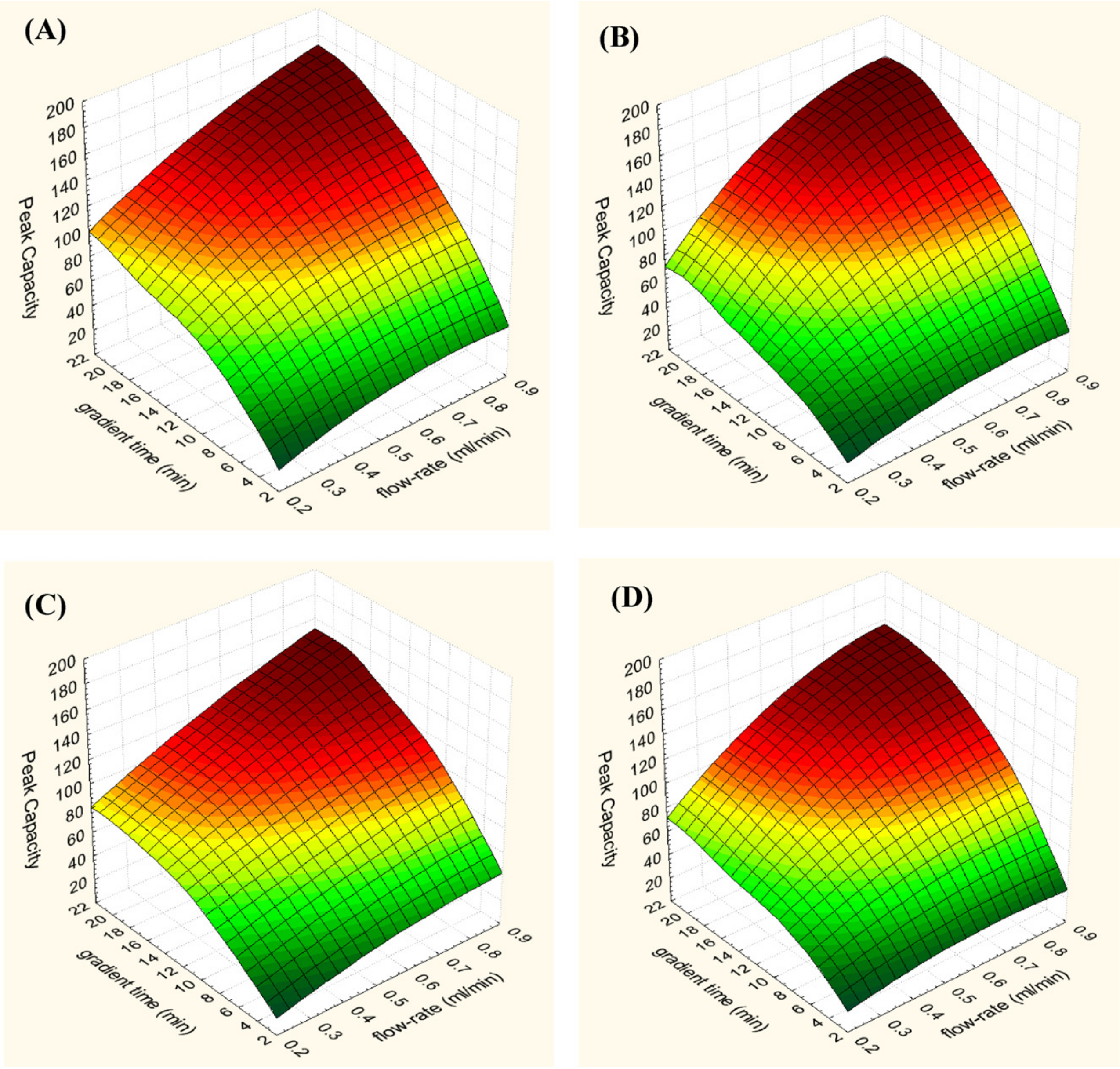

Fig. 3. Peak capacity surface plots as function of flowrate and gradient time. Columns: Kinetex 5 cm × 2.1 mm, 2.6 μm (A), Poroshell 5 cm × 2.1 mm, 2.7 (m (B), Ascentis Express 5 cm × 2.1 mm, 2.7 (m (C) and Waters BEH 5 cm × 2.1 mm, 1.7 (m (D). Conditions: the volume fractions of acetonitrile at the beginning and at the end of the gradient were set constant at 40 and 90%, respectively ($\Delta\varphi = 0.50$), the columns were thermo-stated at 30 °C, the injected volume was 0.5 μl.

packing it was measured previously as 9.5 nm [42]. The advantage of Kinetex column is most significant at 0.3–0.4 ml/min flow-rate. At 0.3 ml/min the Kinetex column performs 15–20% higher peak capacity than the other tested columns. The 5 cm long narrow bore Kinetex and Poroshell columns can generate a peak capacity of 160–170 within 21 min gradient separation when high flow-rates are applied.

Our results confirm the excellent performance of the new columns packed with 2.6–2.7 μm core–shell particles under gradient elution when very fast separations are achieved. For the separation of our pharmaceutical test mix (molecular weight: 270–430 g/mol), the peak capacity of these 5 cm long narrow bore columns is similar or higher to those of the 1.7 μm totally porous particles. For the separation of pharmaceutical compounds by gradient elution, the peak capacities of all four columns significantly decrease with increasing the gradient slope at constant gradient steepness. The highest peak capacity was obtained with the Kinetex column in all cases. It is necessary to emphasize that the efficiency of Poroshell column is very similar to the Kinetex when relatively high flow-rate and long gradient separation is performed. Another finding that the very similar sized ($d_p = 2.7$ μm) and structured ($\rho = 0.63$) new Poroshell-120 and the earlier introduced Ascentis Express particles performs significantly different efficiency in this study. The efficiency of the new generation of sub-3 μm partially porous packings is comparable to those obtained with totally porous sub-2 μm particles in fast gradient separations of pharmaceuticals in the case of short narrow bore columns.

3.5. Peak capacity per unit time and per unit pressure

The column backpressures were also measured and are shown in Fig. 5. The pressure drop characteristics of the columns were examined by subtracting the pressure drop of connection tubes ΔP_{ext} from the total pressure drop ΔP_{tot} obtained with the system to yield

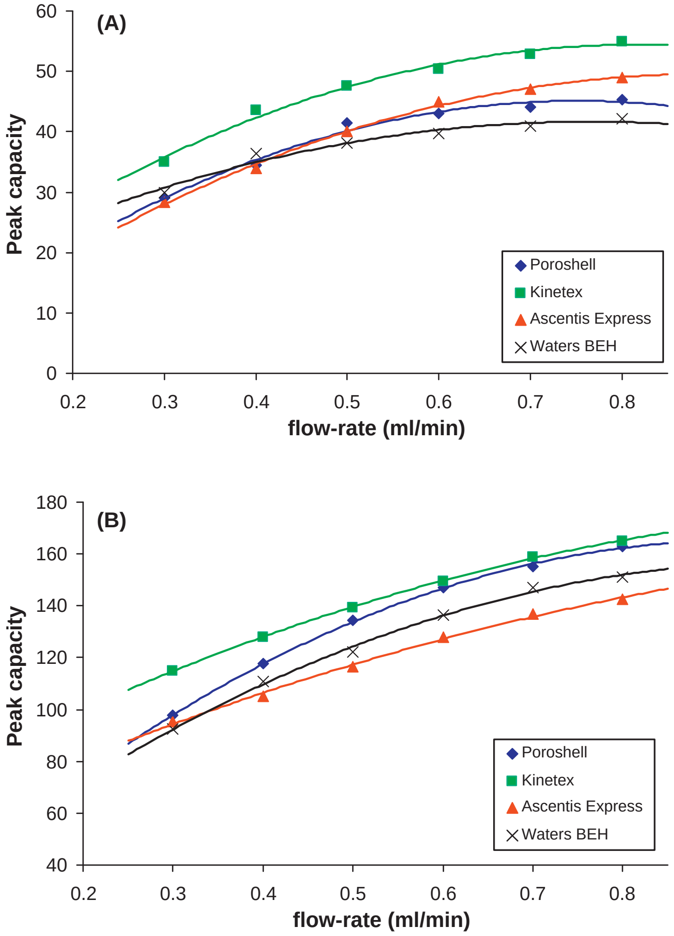

Fig. 4. Peak capacity plots as function of flowrate at 3 min gradient time (A) and at 21 min gradient time (B). Conditions: the volume fractions of acetonitrile at the beginning and at the end of the gradient were set constant at 40 and 90%, respectively ($\Delta\varphi = 0.50$), the columns were thermo-stated at 30 °C, the injected volume was 0.5 µl.

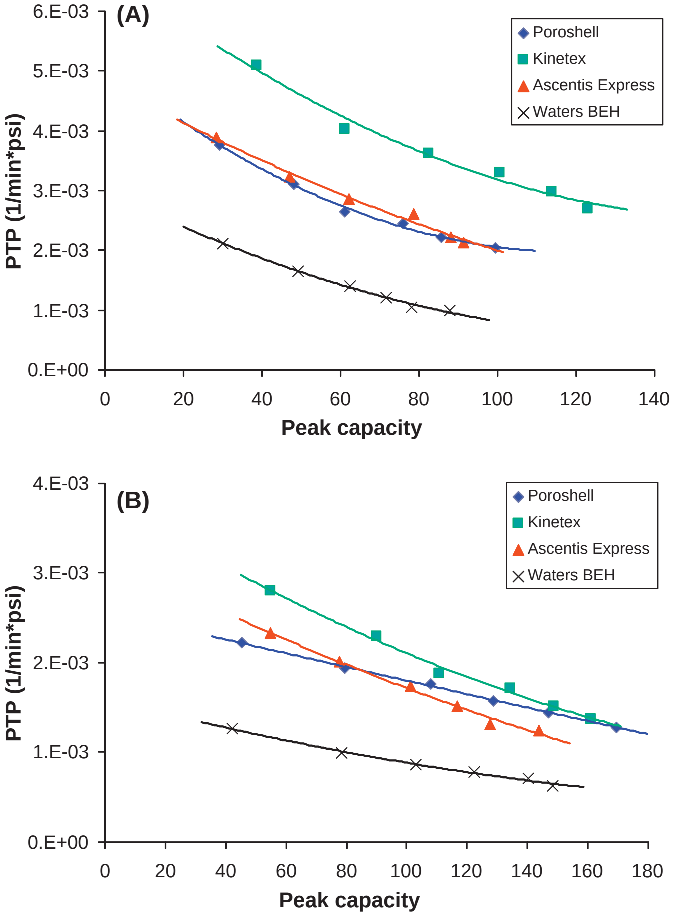

Fig. 6. Peak capacity per unit time and per unit pressure at the flow-rate of 0.3 ml/min (A) and at 0.8 ml/min (B). Conditions: the volume fractions of acetonitrile at the beginning and at the end of the gradient were set constant at 40 and 90%, respectively ($\Delta\varphi = 0.50$), the columns were thermo-stated at 30 °C, the injected volume was 0.5 µl.

the effective column pressure ΔP_{col} ($=\Delta P_{tot} - \Delta P_{ext}$). The extra-column pressure drop was measured with a zero-dead-volume connector instead of the column at each flow rate and the same mobile phase, which was set during the study. For the comparison, the pressure measured at the initial gradient composition was used. These corrected pressure drop values were used to calculate the peak capacity per unit time and per unit pressure (*PTP*). Of course this comparison has some uncertainty since the viscosity of mobile phase changes through the gradient, but in this study the same mobile phase and same gradient program were used for comparing the columns. Therefore we think this comparison gives a correct representation about the possible separation times in gradient elution mode on the four different columns. The measured pressure drop on the sub-2 µm packing is about two times higher than it was obtained with the columns packed with 2.6 and 2.7 µm core–shell particles. Therefore fast gradient separations can be achieved with the 5 cm long narrow bore core–shell columns with the pressure limitation of conventional HPLC systems (400 bar), these columns do not necessitate the use of ultra-high pressure systems.

Fig. 6 shows the obtained peak capacity per unit time and per unit pressure values as a function of peak capacity. On the basis of Fig. 6 it is clear that the number obtained on 2.6–2.7 µm core–shell materials is significantly better than those calculated using sub-2 µm totally porous particles. When slow flow-rate (0.3 ml/min) is applied, the Kinetex column provides the highest *PTP* values (Fig. 6A). The Poroshell and Ascentis Express column performs approximately the 75%, while the Waters BEH column gives only the 40% PTP of Kinetex. When the flow-rate is enhanced to 0.8 ml/min the results are surprising (Fig. 6B). If the peak capacity of 150–180 is required for a separation the Kinetex and Poroshell columns offer practically the same *PTP* values. However if lower peak capacity than 140 is enough for the given separation,

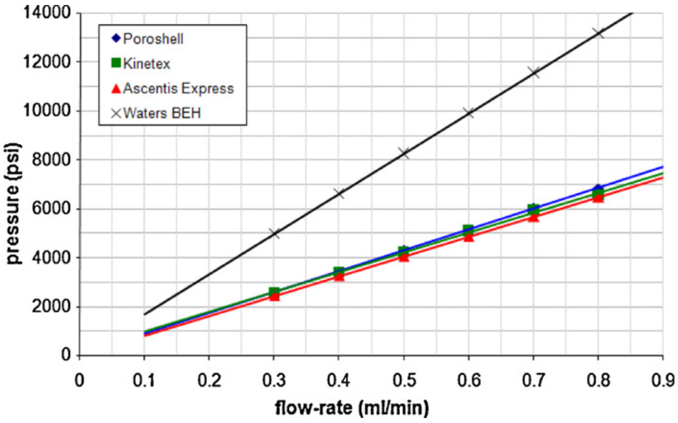

Fig. 5. Column permeability of 5 cm long narrow bore columns. The pressure was measured at the initial gradient composition (40% acetonitrile). The columns were thermo-stated at 30 °C. The column pressure drop was corrected for extra-column pressure drop.

it is better to choose the Kinetex column since the expected analysis time is shorter with the Kinetex column than it is with the Poroshell column. Another interesting result is that if lower peak capacity than 80 is enough, it can be achieved probably faster with the Ascentis Express than with the Poroshell column.

4. Conclusion

Peak capacity of three columns (5 cm × 2.1 mm) packed with 2.6–2.7 μm core–shell particles of different vendors and a column packed with 1.7 μm totally porous particles have been determined and compared as a function of gradient time and flow rate in the case of very fast gradient separations.

All of the three columns packed with core–shell particles offer a really high-separation power in gradient elution with modest operating pressure. For the separation of a pharmaceutical mix (molecular weight: 270–430 g/mol), the peak capacity of these 5 cm long narrow bore columns is similar or higher to those of the 1.7 μm totally porous particles. The highest peak capacity was obtained with the Kinetex column which is in good agreement with the theory. However it was unexpected that as the flow-rate increases, the peak capacity of the Poroshell column ($\rho = 0.63$) is getting closer to that obtained with the Kinetex column ($\rho = 0.73$). The Poroshell column provides 15% less peak capacity at 0.3 ml/min, while at enhanced flow-rate (0.8 ml/min) it performs only 2% less peak capacity than it is in the case of Kinetex.

If the column permeability is considered, the 2.6–2.7 μm core–shell particles offer the possibility of faster gradient separations than it is possible with the 1.7 μm totally porous particles. Surprisingly the very similar sized ($d_p = 2.7$ μm) and structured ($\rho = 0.63$) new Poroshell-120 and the earlier introduced Ascentis Express particles showed different efficiency. Finally we can conclude that the 5 cm long narrow bore columns packed with sub-3 μm core–shell particles offer the chance of very fast and efficient gradient separations in routine pharmaceutical analysis.

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