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The impact of extra-column band broadening on the chromatographic efficiency of 5 cm long narrow-bore very efficient columns

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ABSTRACT

Small columns packed with core–shell and sub-2 μm totally porous particles and monolith columns are very popular to conduct fast and efficient chromatographic separations. In order to carry out fast separations, short (2–5 cm) and narrow-bore (2–2.1 mm) columns are used to decrease the analyte retention volume. Beside the column efficiency, another significant issue is the extra-column band-spreading. The extra-column dispersion of a given LC system can dramatically decrease the performance of a small very efficient column. The aim of this study was to compare the extra-column peak variance contribution of several commercially available LC systems. The efficiency loss of three different type 5 cm long narrow bore, very efficient columns (monolith, sub-2 μm fully porous and sub-2 μm core–shell packing) as a function of extra-column peak variance, and as a function of flow rate and also kinetic plots (analysis time versus apparent column efficiency) are presented.

1. Introduction

Today, there is a need for ultra-fast separations with very high efficiency and sufficient resolution to perform analysis within few minutes. In order to carry out fast separations, short (2–5 cm) and narrow-bore (2–2.1 mm) columns are used to decrease the analyte retention volume and to increase the flow rate [1]. New levels of performance have been achieved with the introduction of very efficient packing materials such as sub-2 μm fully porous particles, silica monolithic rods and core–shell particles. The two current leaders are the sub-2 μm particles [2,3] and the shell particles [4–7], and now columns packed with sub-2 μm core–shell particles are already commercially available [8].

The success of highly efficient, fast separations depends on both column efficiency and on preserving this efficiency by minimizing instrument induced extra-column band spreading. Each serious progress in column technology requires important progress in instrument design and manufacturing [9]. Extra-column band spreading affects the measured performance of columns packed with small particles, especially for columns with an internal diameter smaller than the standard of 4.6 mm [10]. Recently several papers focused on the extra-column effect as a major factor that negatively impacts the apparent performance of columns packed with core–shell or sub-2 μm particles [7–10]. Conventional high performance liquid chromatographic (HPLC) systems contribute by approximately 40–200 μL^2 [9,11] while standard ultra-high pressure chromatographic systems (UHPLC) have a contribution typically in the range of 4–9 μL^2 [7–12]. In the case of very efficient columns, the extra-column variance of the commercially available LC systems with very low dispersion (<10 μL^2) is not negligible. The extra-column peak dispersion of the Waters Acquity system causes an efficiency loss of about 25–35% for the Kinetex 1.7 μm (5 cm × 2.1 mm) column at the optimal linear velocity ($HETP_{min}$) when low molecular weight analytes are separated [8]. Further optimizing UHPLC systems such as using smaller volume needle seat capillary, narrower and shorter connector capillary tubes and a smaller volume detector cell can provide a significant decrease in extra-column contribution down to around 1–5 μL^2 [9,13]. With these improvements the efficiency loss can be significantly reduced [9].

However similar conclusions were provided by Gritti and Guiochon [9,12], this work was to present more practical examples to show the importance of minimizing the extra-column effects. The aim of this study was to compare the extra-column peak variance of two conventional HPLC systems, three hybrid LC systems (which are recommended by the vendors for both conventional and ultra-fast separations) and one UHPLC system with very low dispersion (<10 μL^2) optimized and recommended for ultra-fast separations. The efficiency loss of three different type 5 cm long narrow bore columns (monolith, sub-2 μm fully porous and sub-2 μm core–shell packing) as a function of extra-

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column peak variance is demonstrated. We also calculated the loss in efficiency as a function of flow rate, moreover kinetic plots of analysis time versus apparent column efficiency are also presented.

We used a real life sample in this study. Common test compounds do not reflect the performance attainable for pharmaceutical compounds.

2. Theory

In isocratic elution mode, an additional band broadening, due to the instrumentation (extra-column band broadening), occurs and becomes predominant when the column volume is reduced [10,11]. The measured peak variance (σ^2_{total}) is related to the chromatographic column itself (σ^2_{col}) and all the extra-column volumes and time based contributions of the chromatographic system (σ^2_{ec}). The observed peak variance can be written as:

$$\sigma^2_{total} = \sigma^2_{ec,I} + \sigma^2_{col} + \sigma^2_{ec,D} \tag{1}$$

where the band variances $\sigma^2_{ec,I}$ and $\sigma^2_{ec,D}$ account for the sample dispersion before and after the column, respectively. Extra-column dispersion can be calculated in units of time, volume or column length. Generally the measurement in volume units is preferred.

The extra-column band broadening (σ^2_{ec}) depends on the injected volume, the radius and length of connector tubing, the detector cell volume, the detector time constant, the flow rate, the diffusion coefficient of the sample and on the mobile phase composition. Extra-column peak broadening is usually explained as the sum of volumetric and time-related events [10]. The contribution to the peak broadening of the different parts of the instrument was deeply investigated and presented in several papers [9–12,14]. However it is worthy to mention that various formulas and equations can be found in the literature regarding to the extra-column variance and contribution.

The apparent plate number (N_{app}) contains the contributions of σ^2_{col} and σ^2_{ec}, and can be described, by the following equation:

The apparent plate number (N_{app}) contains the contributions of σ^2_{col} and σ^2_{ec}. The ratio of the apparent and the intrinsic plate number of the column (N_{col}) can be expressed as:

$$\frac{N_{app}}{N_{col}} = \left(\frac{\mu_{1,col} + \mu_{1,ec}}{\mu_{1,col}}\right)^2 \frac{\sigma^2_{col}}{\sigma^2_{col} + \sigma^2_{ec}} \tag{2}$$

where $\mu_{1,col}$ is the column residence time and $\mu_{1,ec}$ is the extra-column residence time.

When the extra-column residence time is much shorter than the column residence time, then the apparent plate number can be written as:

$$N_{app} = N_{col} \frac{\sigma^2_{col}}{\sigma^2_{col} + \sigma^2_{ec}} \tag{3}$$

The contribution of the extra-column volume can simply be considered as an additional constant to the eddy dispersion term, in the Van Deemter equation [14]. Extra-column effects are more significant for scaled down separations (column volume decreases) [10,15,16]. The overall extra-column volume of a Waters Acquity system is about 10–15 μL. This volume represents about 10% of the hold up volume of a 5 cm × 2.1 mm column.

The extra-column peak dispersion of a given instrument (σ^2_{ec}) is generally determined by the systematic measurements of the peak width at half height or by the moment method. The half-height method is based on the following equation:

$$\sigma^2_{ec} = F^2 \frac{\left(t^r_{h,a} - t^f_{h,a}\right)^2}{5.545} \tag{4}$$

where F is the flow rate (expressed in μL/min), $t^r_{h,a}$ and $t^f_{h,a}$ are the rear and front widths of the peak measured at half height obtained by injecting the analyte in the absence of the column. Based on the measurements, σ^2_{ec} in Eq. (4) is given in μL^2. Experimentally observed *HETP* data can be corrected for the contributions of the extra-column volume using the following equation:

$$H = L \frac{\left(t^r_h - t^f_h\right)^2 - \left(t^r_{h,a} - t^f_{h,a}\right)^2}{5.545(t_R - t_a)^2} \tag{5}$$

where t^r_h and t^f_h are the rear and front widths of the peak measured at half height, and t_R and t_a are the elution times (at peak apex) of the test compounds obtained with and without column (respectively).

Beside the half-height method (Eqs. (4) and (5)) another way of calculating the peak variance is the moment method. The band broadening can be derived from the first (μ_1) and the second (μ'_2) central moments which are calculated from the elution peak profiles:

$$\mu_1 = \frac{\int Ce(t)t\ dt}{\int Ce(t)\,dt} \tag{6}$$

$$\mu'_2 = \frac{\int Ce(t)(t-\mu_1)^2\ dt}{\int Ce(t)dt} \tag{7}$$

where $Ce(t)$ is the concentration of the sample compound in the mobile phase leaving from the column as a function of time (t). The values of μ_1 and μ'_2 are calculated by integrating the elution peak profile. The first moment is not always identical to the retention time, however the difference between them is not so significant even when the asymmetry factor is less than 1.5 [14,17]. On the other hand, μ'_2 is equal to the variance of the peak.

The remaining efficiency (E_r) of the column as a function of extra-column variance can be easily calculated according to the next formula:

$$E_r = 100 \cdot \frac{\sigma^2_{col}}{\sigma^2_{total}} \tag{8}$$

The efficiency loss of a column experienced with a given instrument also depends on the mobile phase flow rate. It can be determined by measuring both extra-column peak dispersion and total peak dispersion with a sequence of different flow rates.

Kinetic plots can give an interesting representation of the effect of extra-column variance on the expected analysis time. The effect of extra-column variance on the analysis time can be illustrated by plotting the analysis time or plate-time (t_0/N) against the apparent plate count where apparent plate count is a function of extra-column peak variance (see Eq. (3)). N or N_{app} (if correction for extra-column band broadening is not applied) and t_0 can be calculated according to the following equations introduced by Desmet et al. [18]:

$$N = \frac{\Delta P}{\eta}\left(\frac{K_{V0}}{u \cdot H}\right) \tag{9}$$

$$t_0 = \frac{\Delta P}{\eta}\left(\frac{K_{V0}}{u^2}\right) \tag{10}$$

where ΔP is the available pressure drop, K_{V0} is the column permeability, η is the mobile phase viscosity. Column permeability can be determined experimentally using the following relation:

$$K_{V0} = \frac{u\eta L}{\Delta P} \quad (11)$$

where ΔP is the pressure drop over the column with length L. Viscosity values can be calculated using equations derived by Chen and Horvath [19].

3. Experimental

3.1. Chemicals, columns

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

The test analyte was a small polar neutral pharmaceutical test compound: Estradiol (estra-1,3,5(10)-triene-3,17-diol), produced by Gedeon Richter Plc (Budapest, Hungary). The molecular weight of estradiol is 272 g/mol.

Waters UPLC™ Acquity BEH C18 column with a particle size of 1.7 μm (50 mm × 2.1 mm) was purchased from Waters Ltd., Budapest. Chromolith FastGradient RP-18e column (50 mm × 2.0 mm) was purchased from Merck Ltd., Budapest. The Kinetex Core–Shell column packed with 1.7 μm shell particles (50 mm × 2.1 mm) were obtained from GEN-Lab Ltd., Budapest. All of the columns were still new (no other experiments were performed on them).

3.2. Equipment, software

Measurements were performed using the following HPLC and UHPLC systems: Waters Acquity UPLC system (Waters Ltd., Budapest, Hungary), Agilent 1100 HPLC and Agilent 1200 UHPLC system (Kromat Ltd., Budapest, Hungary), Perkin-Elmer Flexar UHPLC system (Per-form Ltd., Budapest, Hungary), Shimadzu Nexera UHPLC system (Simkon Ltd., Budapest, Hungary), and a Merck Hitachi LaChrom standard HPLC system (Merck Ltd., Budapest, Hungary). All LC instruments were used unmodified, in their standard configuration.

The Waters Acquity system includes a 5 μL sample loop and a 0.5 μL flow-cell. The loop is directly connected to the injection switching valve (there is no needle seat capillary). The tube to the column was 0.13 mm I.D. and 250 mm long, and the capillary located between the column and detector was 0.10 mm I.D. and 150 mm long.

The Agilent 1100 system has a 100 μL sample loop and an 8 μL flow-cell. A standard needle seat capillary tube was used that has 0.17 mm I.D. and 150 mm long. Connector capillaries having 0.17 mm I.D., and 280 mm long (before the column) and 105 mm long (after the column) were used.

The Agilent 1200 system includes a 20 μL sample loop and a 2 μL flow-cell. A small volume needle seat, a 0.12 mm × 100 mm capillary tube was used. Connector capillaries having 0.12 mm I.D., 200 mm long (before the column) and 105 mm long (after the column) were applied.

The Shimadzu Nexera system includes a 20 μL sample loop and a 2.5 μL flow-cell. All capillaries have 0.10 mm I.D. It was 100 mm long at both needle seat and before the column, and 130 mm long after the column.

The Perkin-Elmer Flexar system includes a 50 μL sample loop and a 2.4 μL flow-cell. The needle seat capillary tube has 0.25 mm I.D. and 100 mm long. Connector capillaries having 0.12 mm I.D., and 100 mm long (before the column) and 105 mm long (after the column) were used.

The Merck Hitachi LaChrom system has a 100 μL sample loop and a 15 μL flow-cell. All capillaries have 0.50 mm I.D. It was 150 mm long at needle seat, 500 mm long before the column, and 200 mm long after the column.

Calculation and data transferring to obtain the kinetic plots was achieved by using the Kinetic Method Plot Analyzer template (Gert Desmet, Vrije University Brussel, Belgium). The non-linear curve fitting to plots was performed using MS Excel (Solver).

3.3. Apparatus and methodology

The mobile phase was prepared by mixing appropriate amount of HPLC gradient grade acetonitrile and Milli-Q water. The mixture was degassed by sonication for 5 min. The isocratic mobile phase consisted of 48/52 v/v% acetonitrile/water. Showing the efficiency loss for different retention factors, the mobile phase composition was adjusted to 53/47 v/v% acetonitrile/water and 57/43 v/v% acetonitrile/water (see the caption of Fig. 2).

The stock solutions of reference standards were prepared in acetonitrile (1000 μg/ml). The solutions for the chromatographic runs were diluted from the stock solutions with the mobile phase. The concentration of the test solutions was 10 μg/ml.

The kinetic efficiency (H–u curves) of the three columns was determined earlier and reported in our previous papers [7,8]. The data and constants of previously obtained H–u curves were used in this study.

Six different chromatographs were used in this study for comparing their extra-column variance contribution. The extra-column peak variance of all six investigated LC systems was determined in the same way. It was measured by injecting the test analyte (estradiol) with a zero-dead-volume connector instead of the column at each flow rate and the same mobile phase, which was set during the following flow study. The flow rate of mobile phase was increased from 0.01 ml/min up to 1.2 ml/min. Three parallel injections (1 μL) were performed at each flow rate. Both the moment and half-height method were used for the further calculation. The relative standard deviation of peak widths obtained with three repeated injections did not exceed 5%. The extra-column peak dispersion (σ^2_{ec}) was determined in μL^2 according to Eqs. (4) and (7). It is necessary to mention that calculations of plate height, based on the exact value of second moment (Eq. (7)) of the peaks may be more accurate than values based on half-height method, but they are less precise because of the uncertainty of the estimates of the times when peak integration should start and end, due to the signal to noise ratio experienced with these small sample sizes. Using the moment method is more advantageous when peak profiles are asymmetrical. The plate height values obtained with half-height and moment method were significantly different because of the asymmetrical peak shapes obtained in the absence of a column. The calculation based on the moment method resulted in greater contribution than it was calculated by the half height method. The difference between the results obtained with the two methods was around 10% but in some cases it exceeded 25%.

After determining the extra-column peak variance of the six different LC systems, the maximum efficiency of the three different type 5 cm long narrow bore very efficient columns (Kinetex core–shell C18 1.7 μm, Acquity BEH C18 fully porous 1.7 μm and the monolithic type Chromolith Fast-Gradient) were considered to estimate the possible efficiency loss. Plots of efficiency loss versus flow rate and plots of remaining column efficiency against extra-column variance were calculated.

Kinetic plots were also calculated to show that separation time significantly depends on the extra-column peak variance when very efficient narrow bore columns are used. The data in a mea-

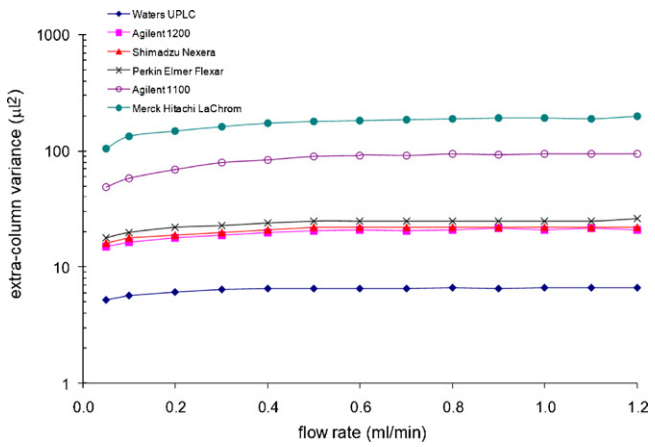

Fig. 1. Plot of extra-column variance versus mobile phase flow-rate. Instrument: Waters Acquity UPLC, Agilent 1200, Agilent 1100, Shimadzu Nexera, Perkin Elmer Flexar, Merck Hitachi LaChrome, mobile phase: 48% acetonitrile–52% water, temperature: 35 °C, injection: 1 μL. A zerovolume union was used in place of the column. Test analyte: estradiol. The possible maximum acquisition rate was set on each instrument (10 Hz on Merck Hitachi LaChrom system, 80 Hz on Agilent 1100 and 1200 systems, and 100 Hz on Shimadzu Nexera, Perkin-Elmer Flexar and Waters Acquity systems).

sured van Deemter curve, apparent plate numbers and the value of the column permeability were used to calculate the kinetic plots (according to Eqs. (3) and (9)–(11)). Data of van Deemter curves are presented in our previous papers [7,8].

4. Results and discussion

4.1. Extra column peak variance of commercially available HPLC and UHPLC systems

The plots of extra-column band spreading (μL^2) as a function of the flow-rate are given in Fig. 1. The possible maximum acquisition rate was set on each instrument (10 Hz on Merck Hitachi LaChrom system, 80 Hz on Agilent 1100 and 1200 systems, and 100 Hz on Shimadzu Nexera, Perkin Elmer Flexar and Waters Acquity systems). The variance of the Acquity system was measured about 6–7 μL^2. This UHPLC system contributed obviously the smallest extra-column variance. The variance of the standard Agilent 1200 and standard Shimadzu Nexera UHPLC systems was measured around 13–20 μL^2, the variance of the standard Perkin-Elmer Flexar system was determined between 18 and 26 μL^2, the variance of the standard Agilent 1100 HPLC system was measured around 50–80 μL^2, while the Merck LaChrom system gave an extra-column peak variance of 100–200 μL^2. The Agilent 1200, Shimadzu Nexera and Perkin-Elmer Flexar system are recommended both for conventional and UHPLC separations, these instruments are so called hybrid LC systems. These hybrid LC systems give approximately 13–26 μL^2 peak variance contribution. The conventional systems have an extra-column peak variance contribution of over 50 μL^2. These differences in extra column peak variance have significant impact on measured column performance, as will be shown later in the paper.

It is important to note that the measured results depend on the sample loop volume, the injection mode, the length and diameter of the capillary tubes and the choice of the detector cell. In this study we used the standard configurations of these LC systems. Further modifications (decreasing the capillary diameter and detector cell volume) can significantly improve the results [9,13].

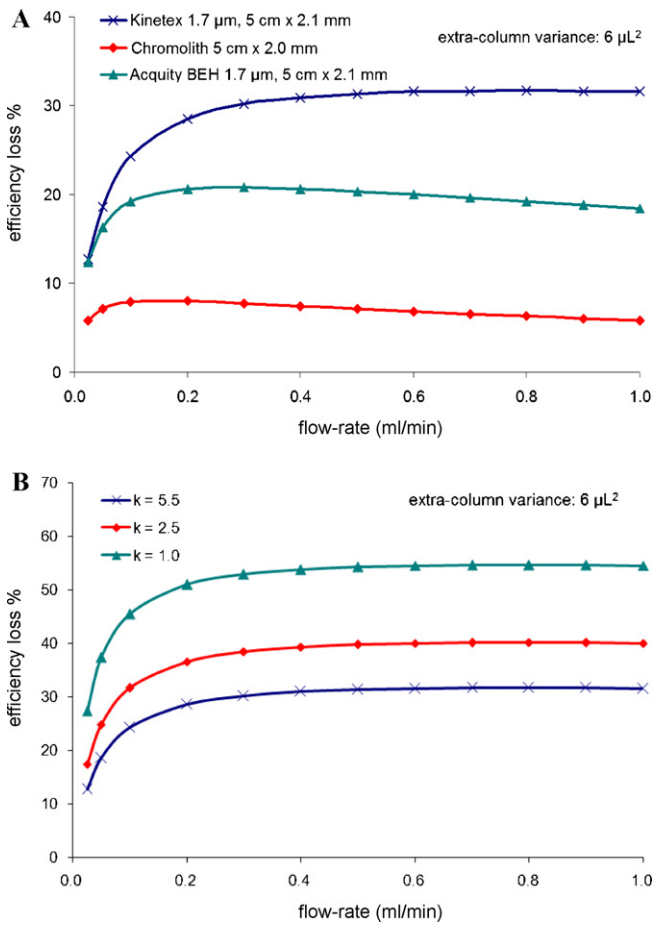

Fig. 2. Efficiency loss versus mobile phase flow-rate. (A) Columns: Kinetex C18 1.7 μm (50 mm × 2.1 mm), Waters BEH C18 1.7 μm (50 mm × 2.1 mm) and Chromolith FastGradient (50 mm × 2 mm), mobile phase: 48% acetonitrile–52% water, temperature: 35 °C, injection: 1 μL. Test analyte: estradiol. Extra-column peak variance: 6 μL^2. (B) Column: Kinetex C18 1.7 μm (50 mm × 2.1 mm), mobile phase: 48% acetonitrile–52% water (k = 5.5), 53% acetonitrile–47% water (k = 2.5) and 57% acetonitrile–43% water (k = 1.0), temperature: 35 °C, injection: 1 μL. Test analyte: estradiol. Extra-column peak variance: 6 μL^2.

4.2. Efficiency loss versus flow rate

In this study, the lowest extra-column variance was obtained with the Waters Acquity system. The contribution of this system to peak variance is about 6 μL^2. This value is in good agreement with previously reported ones [9,10]. This system is recommended for fast separations with narrow bore, short columns. Very surprising results can be obtained when the efficiency loss caused by system dispersion is calculated for 5 cm long narrow bore Kinetex 1.7 μm, Acquity BEH 1.7 μm and Chromolith columns. Fig. 2A shows the efficiency loss as a function of flow rate when extra column peak variance of 6 μL^2 is assumed. The efficiency loss of the given separation was calculated according to the next formula:

$$\text{Efficiency loss} \quad (\%) = 100 - \left[100 \cdot \frac{\sigma^2_{col}}{\sigma^2_{total}}\right] \tag{12}$$

If the most efficient column (Kinetex 1.7 μm) is used, approximately 12–32% of the kinetic efficiency can be lost, by using an optimized UHPLC system with 6 μL^2 peak variance. If the extra column peak variance is 10 μL^2, the efficiency loss reaches 40%, and in the case of an instrument with 50 μL^2 extra column peak variance, the loss in efficiency is approximately 80% (data not shown). A maximum extra-column peak variance of 1.5 μL^2 is allowable with very

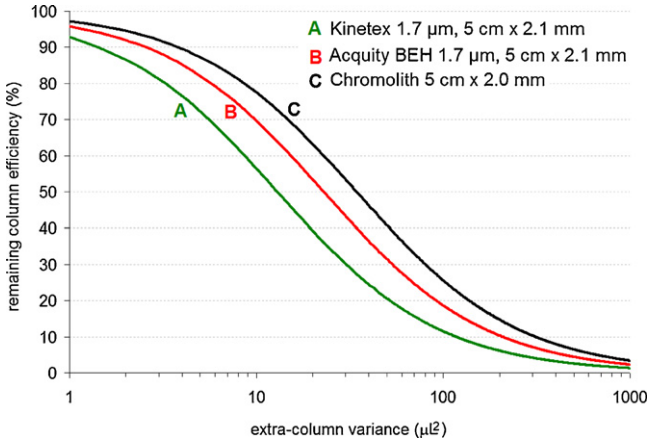

Fig. 3. Plot of remaining column efficiency versus the extra-column variance. Columns: Kinetex C18 1.7 μm (50 mm × 2.1 mm), Waters BEH C18 1.7 μm (50 mm × 2.1 mm) and Chromolith FastGradient (50 mm × 2 mm), mobile phase: 48% acetonitrile–52% water, temperature: 35 °C, injection: 1 μL. Test analyte: estradiol. The peak variance values at maximum column efficiency were considered for the model calculation.

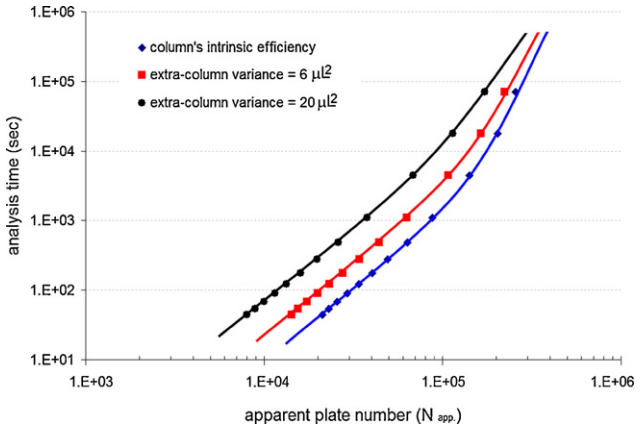

Fig. 4. Analysis time (t) versus apparent plate number (N_{app}) plots of estradiol. Experiments were conducted on a Kinetex C18 1.7 μm (50 mm × 2.1 mm) column, in 48/52 ACN/H_2O, η = 0.85 cP, at 35 °C. Available max. pressure: 1000 bar. Three theoretical cases are calculated for $\sigma^2_{ec} = 0$ μL², $\sigma^2_{ec} = 6$ μL² and $\sigma^2_{ec} = 20$ μL². Retention factor of k = 3 is assumed.

efficient columns when the loss in efficiency is to be kept below 10% (Kinetex 1.7 μm, 50 mm × 2.1 mm column, k = 5, F = 0.2 ml/min).

If we use the Acquity BEH C18 column, we lose about 12–21% of the column efficiency, by using a system with 6 μL² extra column peak variance (Fig. 2). In the case of the monolith column, the efficiency loss is under 10% if use the Waters Acquity system (Fig. 2A). If an instrument with 50 μL² extra column peak variance is used for this narrow bore monolith column, the loss in efficiency is over 50%, at the flow rate of maximum column efficiency (data not shown).

We can conclude that these very efficient, short, narrow bore columns can be used only with significant efficiency loss in recent commercially available UHPLC systems (5–7 μL² extra column peak variance). The loss in efficiency can reach 30% or so.

The influence of the apparent column efficiency of even a small extra-column volume of the instrument used is very important for compounds having low retention factors, which explains why the efficiency of most columns increases with increasing retention factor [14]. It is more important when small columns are used; this is why we have demonstrated data for different k values. Fig. 2B shows the efficiency loss when k is varied between k = 1 and k = 5.5 in the case of Kinetex 1.7 μm column (when extra-column variance is 6 μL²). The loss in column efficiency is about 30% when the retention factor is k = 5.5, it exceeds 40% when k = 2.5 and it goes over 50% when the compound has very low retention factor (k = 1.0).

4.3. Remaining column efficiency as a function of extra-column variance

The apparent column efficiency is function of the "true" column efficiency and of the extra-column band spreading. In the example discussed below the apparent column efficiency is presented as a function of extra-column variance. For the model calculations the maximum column efficiency was considered. The maximum obtainable plate numbers were 19230 with the Kinetex column, 10870 with the Acquity BEH column and 7250 with the Chromolith column (corrected for extra-column variance at optimal flow rate) [7,8]. The remaining column efficiency calculated according to Eq. (8). Fig. 3 shows that, when the most efficient sub-2 μm Kinetex column is used, only 60–90% of the real intrinsic column efficiency can be realized with very low dispersion instruments ($\sigma^2_{ec} < 10$ μL²). When a hybrid system is used, the loss may be as high as 60%. In the case of Kinetex 1.7 μm (50 mm × 2.1 mm) column the use of a hybrid fast LC system is not acceptable. The remaining column efficiency of the Acquity BEH column is higher than that of the Kinetex column. It is possible only with the Chromolith column to utilize the 90% column efficiency with a Waters Acquity system (σ^2_{ec} = 6–7 μL²). In practice, when very efficient small columns are used, optimized chromatographic systems with very low extra column dispersion ($\sigma^2_{ec} < 2$–3 μL²) are mandatory. We can conclude, that redesigned and rebuild UHPLC instruments are necessary to take the full advantage of the most recent very efficient small columns. Today it is not possible to utilize the potential of these small columns.

4.4. Apparent column efficiency, analysis time

Kinetic plots that take into consideration system caused band spreading can be created according to Eqs. (9)–(11), and these plots show the analysis time shift on different LC systems. In this example the analysis time of an analyte (k = 3) was calculated and plotted against the apparent column efficiency. Fig. 4 shows the calculated isocratic kinetic plots obtained with Kinetex 1.7 μm (50 mm × 2.1 mm) column at the maximum applicable pressure (1000 bar) to represent the utilization of maximum performance (UHPLC application). The kinetic plots present three theoretical cases such as $\sigma^2_{ec} = 0$ μL², $\sigma^2_{ec} = 6$ μL² and $\sigma^2_{ec} = 20$ μL². The kinetic plots demonstrate that the additional extra column band broadening of the LC system causes a significant shift upward to longer analysis time. The resulting curves demonstrate the maximum speed obtainable at a given apparent plate number (N_{app}) and also demonstrate the effect of the choice of LC instrument (an optimized UHPLC system with $\sigma^2_{ec} = 6$ μL² and a hybrid LC system $\sigma^2_{ec} = 20$ μL² are compared to the achievable intrinsic column efficiency).

If a given separation requires an apparent plate number of N_{app} = 20,000, it can be performed within 1.5 min on a UHPLC instrument ($\sigma^2_{ec} = 6$ μL²), while the same separation requires 4.6 min analysis time on a hybrid LC system ($\sigma^2_{ec} = 20$ μL²). Theoretically this separation could be achieved within 46 s supposing an LC instrument with zero extra-column band broadening contribution. So, we can state that UHPLC instruments with very low dispersion have a serious impact on the achievable analysis time if very efficient small columns are applied. Further improvements in instrument design (smaller dispersion) can drastically shorten

the analysis time, utilizing the performance of the most recent commercially available columns.

5. Conclusion

The aim of this study was to compare the extra-column peak variance contribution of several commercially available LC systems. According to the results, recent LC systems can be classified in three groups: (1) optimized systems for fast separation with very low dispersion ($\sigma^2_{ec} = 10\ \ \mu L^2$), (2) hybrid LC systems recommended for both fast and conventional separations ($\sigma^2_{ec} = 10–30\ \ \mu L^2$) and conventional LC systems with an extra column variance over 50 μL^2. These major differences in extra column peak variance have a significant impact on measured column performance and achievable analysis time.

The efficiency loss of three different type 5 cm long narrow bore, very efficient columns (monolith, sub-2 μm fully porous and sub-2 μm core–shell packing) as a function of extra-column peak variance, and as a function of flow rate and also kinetic plots (analysis time versus apparent column efficiency) were presented.

According to this study, we can conclude, that further improvements in instrument design (smaller dispersion) are necessary to take the full advantage of the most recent very efficient small columns. Today it is not possible to utilize the potential of these small columns. The loss in efficiency can reach 30–55% with commercially available optimized UHPLC systems.

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