Advances and Challenges in Extremely High-Pressure Liquid Chromatography in Current and Future Analytical Scale Column Formats

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Abstract
The present study speculates on the potential gain we can expect of a further leap in pressure by moving from the current 1500 bar to a futuristic 3000 bar, as well as reviews the main impediments to be expected when trying to realize such systems. The study focuses on so-called “analytical scale” separations, i.e., separations that are currently carried out in 2.1 mm columns, as this is the current state-of-the-art in UHPLC instrumentation.

Introduction
Since the advent of HPLC, the improvements in column packing materials and instrumentation have increased the achievable separation efficiency from a few hundred to a few hundreds of thousands of theoretical plates and decreased analysis times from hours down to seconds [1-6]. Considering chromatographic columns can maintain the same packing quality and structure when particle size is varied, in many cases a good first approximation, the performance kinetics in HPLC are straightforward as the plate height $H$ is linearly proportional to the particle size $d_p$ while the optimal velocity $u_{opt}$ varies in an inversely proportional way [7]. As a result, a reduction of particle size by factor of two doubles the separation efficiency $N (N=H/L)$ and halves the analysis time $t$. However, when looking at the relationship describing the pressure drop $\Delta P$ over a column with a certain flow resistance $\phi$ and viscosity $\eta$, generally referred to as the Kozeny-Karman equation:

$$\Delta P_{col} = \frac{u_0^2 \eta \phi_0}{d_p^2}$$

(1)

it is found that this particle size reduction comes at the cost of an increase in pressure by a factor of 8 (assuming operation at $u_{opt}$, since also $u_{opt}$ doubles when $d_p$ is halved). Even if one would consider the same separation efficiency by reducing the column length $L$ by factor of two, still an increase in operation pressure by a factor of 4 would be required. This trade-off between particle size, column length and available operating pressure is commonly described by the so-called Knox-and-Saleem equation describing the minimum analysis time $t_R$ or column void time $t_0$ ($t_R = [1+k] \cdot t_0$) to obtain a given efficiency [8,9]

$$t_0 = \frac{h_{min^2} \eta \phi}{\Delta P_{max}} \cdot N^2$$

(2)
To obtain this efficiency, one needs to operate the ideal particle size and column length for the desired efficiency or analysis time. These optimal values are given by Eqs. (3a) for a given efficiency and by Eq. (3b) for a given column void time $t_0$:

$$d_p = \sqrt{\frac{\eta \cdot D_{mol} \cdot h_{min} \cdot v_{opt} \cdot \theta}{\Delta P_{max} \cdot N}} \quad (3a)$$

$$d_p = \sqrt{\frac{4 \cdot \eta \cdot D_{mol}^2 \cdot v_{opt}^2 \cdot \theta}{\Delta P_{max} \cdot t_0}} \quad (3b)$$

where $v_{opt}$ is the optimal reduced velocity and $D_{mol}$ the diffusion coefficient. The corresponding optimal column lengths are obtained as the relationship between column length and plate height as $L_{opt} = h_{min} \cdot N = h_{min} \cdot d_p \cdot N$. Figures 1a and 1b show plots of the optimal particle size and column length as a function of the maximum operating pressure for four different efficiencies. Two trends are obvious from these plots. Firstly, for a higher efficiency, longer columns packed with larger particles are to be preferred over smaller particles in short columns. Secondly, the optimal particle size and columns lengths decrease continuously with increase operating pressure, as could be expected from Eq. (3a), showing $d_p \sim 1/\Delta P_{max}^{0.5}$. A very similar trend is found when considering different analysis times rather than $N$ values, although here a $d_p \sim 1/\Delta P_{max}^{0.25}$-relationship is found (see Eq. 3b). These relationships show how the increase in operating pressure in LC that came with the advent of the UHPLC instrumentation goes hand in hand with the use of smaller particles and shorter columns. In this work, the focus will be on packed bed columns since they still make up the vast majority of the LC market for normal and narrow bore columns. Monolithic or chip based columns are currently only competitive with packed bed columns in capillary or nano-LC systems.

Figure 2 provides a quantitative view on the gain in analysis time and efficiency that has been realized when going from the pre-UHPLC era (blue curves) to today’s state-of-the-art, with UHPLC instrumentation offering pressures up to 1500bar and with core-shell particle columns reaching reduced plate heights around or below 1.5 (red curves). Whereas Fig. 2a represents the more customary kinetic plot of time versus $N$ ($t_0$-time in the present case), the same data are replotted in Fig. 2b where the y-axis now represents the impedance time $t_0/N^2$. The impedance time is the natural parameter to “normalize” the time against the required efficiency (as can readily be seen by reshuffling Eq. (2)) and provides the clearest possible zoom on the differences in required analysis time [9,10]. As can be noted when comparing the
best possible systems of each era, the gain in analysis time resulting from the introduced improvements is on the order of a factor of 10.

Important to note is that the introduction of the UHPLC technology not only changed the pressure but also the column diameter. As the heat generated inside the columns is proportional to the operating pressure and flow rate, which both increase when moving to UHPLC, the column diameter had to be reduced (from 4.6mm to 2.1mm) to counter this effect. In addition, the lower flow rates of these narrow bore columns improved compatibility with MS detection. The strong reduction in column volume however required a concomitant reduction in extra-column dispersion contribution to avoid excessive loss in separation efficiency. Whereas a typical 400bar instrument displays a system band broadening on the order of \( \sigma_{V,\text{extra}}^2 \approx 25 \ \mu \text{L}^2 \), the best state-of-the-art UHPLC instruments typically only have a \( \sigma_{V,\text{extra}}^2 = 2.5 \ \mu \text{L}^2 \). If the contributions from extra-column band broadening or viscous heating would not be taken into account, all curves in Fig. 2b would reach the same minimum value equal to \( h_{\text{min}}^2 \cdot \eta \cdot \phi / \Delta P_{\text{max}} \) (see Eq.(2)) and the envelopting curves shown in Figs. 3a and 6 further on would be horizontal lines. The upward shift of this minimum in the low N-range, corresponding to shorter columns and smaller particles, is thus a measure for the instrumental and viscous heating induced losses in separation performance.

In the present contribution, we speculate on the possibility and suitability (=return on investment) of a further increase in pressure, say by going from 1500 to 3000bar. As it is difficult to answer the suitability question in hard economic terms, the present contribution merely aims at quantifying the potential theoretical increase in efficiency and speed that would be achievable when performing packed bed LC separations at 3000bar. As shown in the Theory and assumptions section, this prediction is calculated using the well-established kinetic performance equations of chromatography and a best-guess estimate for the two most important aspects that can be expected to impede successful 3000bar separations, i.e., the effects of viscous heating and extra-column band broadening. Although the analysis has been performed for isocratic separations by representing N values, the gradient separation performance follows a similar trend since the obtained peak capacity is proportional to \( N^{0.5} \) and a constant factor determined by the sample properties and mobile phase gradient (assuming a constant ratio of gradient time \( t_G \) and \( t_0 \)). In that case, the SG-factor derived
further on represents the time gain that can be achieved for a given peak capacity rather than for a given efficiency.

**Theory and assumptions**

We assume the intrinsic band broadening in the columns can be described by the well-established van Deemter-equation:

\[ H_{\text{col}} = A \cdot d_p + \frac{B \cdot D_{\text{mol}}}{u_0} + \frac{C \cdot u_0}{D_{\text{mol}}} \cdot d_p^2 \]  
(4)

Assuming a state-of-the-art packing quality, typical parameter values are \( A=0.9, B=3 \)
(assuming a component with retention factor around \( k=3 \)) and \( C=0.1 \) for fully-porous particles. For core-shell particles, these values become: \( A=0.65, B=2.5 \) and \( C=0.075 \). Flow resistance values were taken as \( \phi_0=800 \) for fully-porous particles and \( \phi_0=650 \) for core-shell particles and the total column porosity was set at \( \varepsilon_T=0.55 \). Next to this basic peak broadening contribution (flow and mass transfer), peaks are also broadening in the flow circuitry outside the column (injector, connection tubing, detector). This so-called instrument band broadening has been well studied in the past [11-22] as well as in recent years [5,23,24-37]. In the present contribution, we suffice by lumping all contributions into a single extra variance contribution \( \sigma^2_{V,\text{extra}} \). As a rule-of-thumb, \( \sigma^2_{V,\text{extra}} \) varies with a 4th order (!) dependency on the diameter of the flow conduits (connection tubing, internal flow-through parts of the injector or the detector cell), while it only varies linearly or quadratically with the length of these items. This shows that a reduction of the internal diameter of the different instrument parts is always much more effective to limit \( \sigma^2_{V,\text{extra}} \) than a reduction of their length.

However, when considering a pressure-drop limited context, it is also important to realize that a reduction of the internal diameter also leads to a 4th power increase of the pressure losses.

To simplify the pressure loss calculation, the extra-column volumes making up the “system” have in the present study been represented as a uniform piece of tubing with given length and diameter, such that:

\[ \Delta P_{\text{extra}} = 128 \cdot \frac{F \cdot \eta \cdot L_{\text{tub}}}{\pi \cdot d_{\text{tub}}^4} \]  
(5)
This approach negates the presence of the turns and constrictions that are inevitably present in any practical system, but offers the advantage of simplicity. Taking $d_{\text{tub}}$=75$\mu$m and $L_{\text{tub}}$=60cm to represent the state-of-the-art in the current generation of UHPLC instruments, this approach leads to values of $\Delta P_{\text{extra}}$ that are indeed typically encountered in practice, which are of the order of 52bar to 258bar in the 0.4 to 2.0 mL/min range (viscosity $\eta$=10$^{-3}$Pas$\cdot$s). For the future extreme-low dispersion instruments needed when moving to 3000bar (cf. Figs. 5-6 further on), it was assumed that $d_{\text{tub}}$=35$\mu$m (to sufficiently decrease $\sigma^2_{V,\text{extra}}$) and $L_{\text{tub}}$=10cm. This length is very short (especially considering it also includes the injector and detector circuitry), but is needed to keep the pressure losses (increasing with $d_{\text{tub}}$4) within reasonable limits. Remark that $L_{\text{tub}}$=20cm would also still be permissible (data not shown).

Another major source of band broadening emerging at elevated pressures results from the viscous heating effect [38-54]. In the present study, all calculations are based on the general expression derived in [55] to represent the long-time limit viscous heating contribution:

$$H_{VH} = \frac{1}{2\kappa} \cdot \Delta v \cdot u_0 \cdot \frac{d^2_{\text{col}}}{D_{rad}} \cdot \Delta \mu,$$

(6)

where $\kappa$ is a geometrical constant from Aris-Taylor method [55,56], $D_{rad}$ the radial dispersion coefficient in the packed bed (set at 2$\cdot$10$^{-9}$m$^2$/s) and $\Delta v$ the velocity difference between the center and wall of the column relative to the average velocity. Using in-house simulations of the radial temperature-profiles metal columns in a still-air, the average value for $\Delta u$ and $\kappa$ could be obtained. Taking the limiting case of a fixed wall temperature (isothermal conditions), the radial temperature profile is given by

$$T(r) = T_w + \frac{q \cdot d^2_{\text{col}}}{16 \cdot \lambda} \cdot \left(1 - \frac{r^2}{R^2_{\text{out}}}\right),$$

with

$$q = F \cdot \Delta P \cdot (1 - \alpha \cdot T) = \left(\frac{\Delta P}{L}\right)^2 \cdot \frac{\epsilon d^2_{\text{tub}}}{\eta \phi_0} \cdot (1 - \alpha \cdot T)$$

(7)

Herein is $\lambda$ the thermal conductivity of the packed bed taken as 0.55 W/mK [57] and $\alpha$ the isothermal compressibility of the mobile phase. The temperature dependency of the viscosity and $\alpha$ were modelled after experimental data obtained at a pressure of 1000bar for an acetonitrile/water mixture 50/50 V% [58]. Eq. (7) shows that the expected radial temperature and velocity profiles significantly increase with column diameter and operating pressure.
expression in Eq. (6) is then combined with the general transient factor accounting to also calculate the correct contribution in short length columns [1,23,35, 59]:

\[ H_{VH} = H_{VH,\infty} \cdot \left[ 1 - \frac{u_0 d_{col}^2}{a D_{rad} L} \cdot \left( 1 - e^{-a D_{rad} L/(u_0 d_{col}^2)} \right) \right] \]  

(8)

where \( a \) is a geometrical factor determined by the shape of the velocity profile [59]. Note this approach neglects the effect of the retention enthalpy which will inevitably induce an additional radial velocity difference aggravating the effect.

With the aforementioned band broadening sources, and knowing that \( \sigma_{V,\text{col}}^2 = H_{VH} \cdot L \cdot A_{\text{col}} \cdot \epsilon_T \cdot (1+k) \) with \( A_{\text{col}} \) the column cross-section and \( \epsilon_T \) the total column porosity (consistently put at 0.55 in the present study), we can introduce a corrected (i.e., total) plate height \( H_{\text{tot}} \):

\[ \sigma_{V,\text{tot}}^2 = \sigma_{V,\text{col}}^2 + \sigma_{V,\text{extra}}^2 + \sigma_{V,VH}^2 \]  

(9a)

\[ H_{\text{tot}} = H_{\text{col}} + \frac{\sigma_{V,\text{extra}}^2}{A_{\text{col}} \cdot \epsilon_T^2 \cdot L \cdot (1+k)^2} + H_{VH} \]  

(9b)

With the above, all expressions are available to establish kinetic performance limit plots, representing the minimal required analysis time as a function of the desired number of theoretical plates. The procedure has been programmed in Matlab and uses the value of the column diameter and a pre-selected series of \( u_0 \)-values to calculate the corresponding flow rates. These are then used in (5) to calculate the extra-column pressure-drop \( \Delta P_{\text{extra}} \) for each \( u_0 \)-value. Once these values are known, the available column pressure drop can be directly calculated as \( \Delta P_{\text{col}} = \Delta P_{\text{max}} - \Delta P_{\text{extra}} \), with \( \Delta P_{\text{max}} \) the maximal available instrument pressure. Subsequently using the \( \Delta P_{\text{col}} \)-values in Eq. (1), we can calculate the different column lengths \( L \) that go with each of the selected \( u_0 \)-values. With \( L \) and \( u_0 \) known, all the ingredients are then available to compute the effective plate height \( H_{\text{tot}} \) via Eq. (9b). Finally, using the known \( L \) together with the identities \( L=N \cdot H_{\text{tot}}=u_0 \cdot t_0 \), a \( (t_0,N) \)-data couple can be calculated for each considered \( u_0 \)-value and the pursued kinetic plot can be established.

An extra routine has been added to the program to compute the enveloping curves shown in Figs. 3a and 6 and used to compute the speed gain factors \( SG \) (see Eq. 10 further on). Briefly, the enveloping curves were obtained by calculating the kinetic performance limit curves for a
wide range of particle sizes (from 0.25µm to 4µm with intervals of 0.05µm) and subsequently
determining the minimum $t_0/N^2$ for each $N$-value.

**Potential gain, requirements and design of a future 3000bar system**

With the above equations, we are now ready to extrapolate the current 1500bar UHPLC
performance to that of a hypothetical, future 3000bar system. Fig. 3 shows the potential gain
in analysis time one can expect when moving to a 3000bar instrument, assuming all other
conditions (column diameter, the thermal conditions, system dispersion) remain the same. To
obtain the best possible zoom-in on the difference, again the $t_0/N^2$-representation is preferred
(Fig. 3a). To quantify the gain in analysis time across the entire $N$-range, a speed gain factor
SG can be defined as:

$$SG = \frac{t_{3000\text{bar}}}{t_{1500\text{bar}}} \quad (10)$$

wherein $t_{1500\text{bar}}$ and $t_{3000\text{bar}}$ are taken from the enveloping curves added to Fig. 3a. These curves
describe how the minimal required $t_0$-time varies with $N$ assuming all operational parameters
(mobile phase velocity, column length, pressure drop) are optimized and assuming each $N$ is
pursued with its individually optimized particle size (see also Eq (3a)).

Representing the variation of the SG factor with $N$,Fig. 3b shows that, under the prevailing
assumptions, a move to a 3000bar operation would only start to be advantageous (SG>1) for
separations requiring $N>20,000$ plates. And it is only for separations requiring $N>50,000$ plates
that the speed increase would be larger than 50%. In the large $N$-limit, the SG-curve nicely
converges to the factor of 2 that can be expected based on Eq. (2). In the absence of any
significant viscous heating or extra-column band broadening or pressure drop, as is the case
in the high $N$-range given the large involved column lengths, all parameters in Eq. (2) remain
the same when going from 1500 to 3000bar. Except of course the value of $\Delta P_{\text{col}}$, which
doubles, thus explaining the expected factor of 2. The fact that the actual value is somewhat
larger than 2 is due to the fact that the 1500bar reference case is even at $N=200,000$ still
slightly affected by extra-column pressure losses.

The counterproductive effect of the 3000bar operation in the $N<20,000$ plate region can be
largely attributed to the larger viscous heating compared to the 1500bar case. Viscous heating
essentially affects the speed of low efficiency separations because, the shorter the required
column, the larger the pressure gradient $\Delta P_{\text{col}}/L$, which in turn is the factor determining the
generated friction heat (see Eq. (7)). The SG≈2 for the high efficiency region illustrates that
the applicability of instruments with a higher operation pressure would currently be limited
to very high efficiency separations where long, coupled columns are used that can more
efficiently dissipate heat and are less affected by extra-column band broadening effects due
to their large volume [60].

To further investigate the reasons for the adverse effect of a higher pressure on separation
speed, Fig. 3b also contains three hypothetical curves, one where the viscous heating is
switched off, one where the extra-column dispersion and pressure losses are switched off and
one wherein both features are combined. As can be noted, the effect of the extra-column
dispersion contributions (dashed line) is small, while the ability to eliminate the viscous
heating band broadening (dotted line) would provide gain factors around and above the factor
2 expected on the basis of Eq. (2)), confirming the earlier statement on the reasons for the
SG<1-values in the N<20,000-range. Combining both effects (no viscous heating or instrument
losses; dash-dotted line), the SG-value steeply increases with reducing N, because the
reference 1500bar separation itself is also already strongly limited by extra-column dispersion
in the low N-range and, to a minor degree, by viscous heating effects.

Unfortunately, there are currently no good solutions within grasps that would allow to sup-
press viscous heating to the level where its contribution to band broadening would become
insignificant, although some potentially workable ideas have been circulating, such as the use
of particles with enhanced-conductivity cores [57], the use of metal-core monolithic columns
[57] or the use of column tube walls with a restricted axial heat conductivity, the improvement
of the adiabatic insulation conditions [53], or a combination of all of the aforementioned.

Since the hypothetical cases considered in Fig. 3b still seem very far away, the only current
viable option to reduce viscous heating band broadening is to reduce the column diameter (cf.
the $d_{col}^6$-dependency in Eq. 6), albeit that it still needs to be demonstrated that sub-2mm
columns can be packed with the same quality as the current 2.1mm columns, given the
fundamental doubts that can be raised on this point [61].

The effect of $d_{col}$ is investigated in Fig. 4. As can be noted, only the 1.8mm column can be
expected to give a minor improvement in the practically interesting region of 10 to 30,000
plates, while the smaller column diameters all perform worse than the standard 2.1mm,
Despite their much smaller viscous heating contribution. The poor performance of the smaller column i.d.’s can be attributed to the fact that these are more prone to the extra-column dispersion since the column contribution to the total dispersion in Eq. (9) scales with $d_{col}^4$.

To investigate this, Fig. 5a considers the same range of column diameters, but now assuming an instrument with a 10-fold smaller dispersion ($\sigma_{V,extra}^2=0.25\mu L^2$). As can be noted, all speed gain curves now lie above the unity level. However, the speed gain in the practically most relevant 10 to 30,000 plate range is still very modest (factor 1.6 to 1.8 gain) for the best solution ($d_{col}=1.2mm$). This is due to the fact that, while the smaller column i.d. is highly effective to suppress the viscous heating contribution, the smaller column cross-section (>4 times smaller than 2.1mm column) puts extra demands on the extra-column dispersion. This is illustrated in Fig. 5b, where we assume an instrument that has a 50-fold smaller dispersion ($\sigma_{V,extra}^2=0.05\mu L^2$) than the current standard. Now the curves roughly lie in order of increasing diameter (smallest i.d. the highest), i.e., in order of increasing viscous heating contribution, with the highest curve (0.9mm i.d. column) consistently above the factor 2 speed gain.

Finally, Fig. 6 shows the kinetic performance plot of the best possible 3000bar separation selected from all cases considered in Figs. 3 and 5 and compares them with the current state-of-the-art. The selected system requires a $\sigma_{V,extra}^2=0.05\mu L^2$ and uses $d_{col}=1mm$ columns (the latter turned out to give a slightly better performance in the practically most relevant range of N=10 to 30,000 plates than the 0.9 and 1.2mm columns considered in the previous figures). It should be noted that if it would be possible to reduce the contribution of $\sigma_{V,extra}^2$ to such a small value, also the effect from the column hardware (flow distributor and inlet frit) should be minimized, as these play a similar role in the overall separation performance. It was for example found in a recent study that a single inlet could contribute up to 0.6µL² to the column peak variance for a 2.1mm ID column [62].

Concluding remarks

The above considerations have made clear that the gain to be expected from extreme high pressure separations at say 3000bar is relatively small: a factor of 2 gain in separation speed at most. This is much smaller than the gain made in the mid 00’s with introduction of UHPLC and core-shell particles, while yet still many developments are needed: the production of well-packed 1 or 1.2mm columns, the production of uniform and mechanically robust 1µm
particles, and the design instruments and detectors with a 50-fold (!) reduction of $\sigma_{V,\text{extra}}^2$.

Notably the last requirement seems to be very demanding, especially when considering that most detector schemes tend to lead to poorer S/N-ratio’s when their volume or measurement time frame is shrunk.

Other factors impeding the development of a commercially viable 3000bar extreme high-pressure separation system are the current lack of good pressure-compliant column housing and connectors, the need for pressure-compliant flow meters capable of the required precision, while also a new generation of rotor-stator valves will need to be developed. Undoubtedly, the wear of these and other instrument will add to their production and maintenance cost, while users will also have to cope with the retention equilibrium shifts that can be expected at these extreme pressures when trying to translate their methods. Other physicochemical factors compromising the conduction of extreme high-pressure separations are the increased viscosity of the mobile phases and concomitant reduction in diffusion coefficient, increase in melting point (e.g. 20°C for acetonitrile at 3656bar) and (reversible) deformation of the column housing and particles that might affect bed stability and porosity [63].

The application range where extreme pressure separations might see their first appearance is most likely to be found in the ultra-high efficiency separations, where viscous heating and extra-column dispersion and pressure losses are minimal, because of the large column lengths leading to correspondingly low pressure gradients ($\Delta P/L$) and large column volumes. In that area, it would be possible to do N=100,000 plate separations in a total analysis time of 10min (last component eluting at k=10), which is the time that was needed to for just an N=20,000 plate separation in the pre-UHPLC era.

References


Figure captions

**Figure 1.** Plots of (a) the optimal particle size and (b) corresponding optimal column length as a function of the maximum operating pressure for four different efficiencies (top to bottom: N = 100,000; 40,000; 20,000 and 10,000), calculated using Eq. (3) assuming $h_{min}=1.6$, $D_{mol}=10^{-9} m^2/s$, $\eta=10^{-3} Pa\cdot s$, $\phi=650$, $\nu_{opt}=5.8$. These conditions are representative for small molecule separations under standard temperature conditions (30°C) and mixtures of water-acetonitrile as mobile phase.

**Figure 2.** Kinetic performance plot comparison of $\Delta P_{\text{max}}=400$bar-instruments with $\sigma_{V,\text{extra}}^2=25 \mu L^2$ and columns with $d_{col}=4.6$mm packed with fully-porous particles ($d_p=5 \mu m$; $d_p=3.5 \mu m$ and $d_p=1.7 \mu m$, $\phi=800$, blue curves) and $\Delta P_{\text{max}}=1500$bar with $\sigma_{V,\text{extra}}^2=2.5 \mu L^2$ using columns with $d_{col}=2.1$mm packed with core-shell particles ($d_p=2.7 \mu m$; $d_p=2.0 \mu m$ and $d_p=1.3 \mu m$, $\phi=650$, red curves). (a) plot of $t$ versus N; (b) corresponding plot of $t/N^2$ versus N.

**Figure 3.** Kinetic performance plot comparison of today’s state-of-the-art (1500bar, red curves, see caption of Fig. 2 for details) and a hypothetical $\Delta P_{\text{max}}=3000$bar instrument with $\sigma_{V,\text{extra}}^2 (=2.5 \mu L^2)$ using standard 2.1mm columns packed with core-shell particles with diameter $d_p=2.0 \mu m$; $d_p=1.3 \mu m$ and $d_p=1.0 \mu m$ (blue curves). (a) plot of $t_0/N^2$ versus N with enveloping curves (bold dashed lines); (b) corresponding plot of the speed gain factor $S_G$ (full line) based on the enveloping curves added to (a), and for three hypothetical cases: $H_{VH}=0$ (dotted line), $\sigma_{V,\text{extra}}^2=0$ (dashed line) and both effects combined (dash-dotted line). Same physicochemical parameters as in Fig. 2.

**Figure 4.** Evolution of speed gain factor $S_G$ with N for 5 different $d_{col}$-values: 2.1mm (blue), 1.8mm (green), 1.5mm (red), 1.2mm (magenta) and 0.9mm (black). All other parameters identical to Fig. 3.

**Figure 5.** Effect of extra-column dispersion on the evolution of speed gain factor $S_G$ with N for 5 different $d_{col}$-values: 2.1mm (blue), 1.8mm (green), 1.5mm (red), 1.2mm (magenta) and
0.9mm (black) \( \sigma^2_{V,\text{extra}}=0.25\mu L^2 \) and (b) \( \sigma^2_{V,\text{extra}}=0.05\mu L^2 \). All other parameters identical to Fig. 3.

**Figure 6.** Kinetic performance plot comparison of today’s state-of-the-art (1500bar, red curves, see caption of Fig. 2 for details) and best conceivable \( \Delta P_{\text{max}}=3000\text{bar} \)-instrument with \( \sigma^2_{V,\text{extra}}=0.05\mu L^2 \) and using optimally-packed 1mm columns with core-shell particles with diameter \( d_p=2.0\mu m; d_p=1.3\mu m \) and \( d_p=1.0\mu m \) (blue curves).

For Table of Contents Only:
Analysis time

Separation Efficiency

400 bar
1500 bar
3000 bar
Figure 1:

(a) $d_p \text{ (\(\mu\text{m}\))}$ vs. $\Delta P_{\text{max}} \text{ (bar)}$

- $N = 100,000$
- $N = 40,000$
- $N = 20,000$
- $N = 10,000$

(b) $L \text{ (cm)}$ vs. $\Delta P_{\text{max}} \text{ (bar)}$

- $N = 100,000$
- $N = 40,000$
- $N = 20,000$
- $N = 10,000$
Figure 2:

(a) $t_0$ (s) vs. $N$

(b) $t_0/N^2$ (s) vs. $N$
Figure 3:

(a) 

(b) 

$\frac{t_0}{N^2}$ (s) 

$N$ 

$SG$ 

$N$ 

$H_{VH} = 0$

$\sigma^2_{V,extra} = 0$

$H_{VH} = 0$

$\sigma^2_{V,extra} = 0$
Figure 4:

$\text{SG}$

$d_{\text{col}} = 0.9\text{mm}$

$d_{\text{col}} = 2.1\text{mm}$

$d_{\text{col}} = 1.8\text{mm}$

$d_{\text{col}} = 1.5\text{mm}$

$d_{\text{col}} = 1.2\text{mm}$
Figure 5:

(a) $SG_{ndcol}=0.9\text{mm}$

(b) $SG_{ndcol}=2.1\text{mm}$

- $d_{col}=1.5\text{mm}$
- $d_{col}=1.2\text{mm}$
- $d_{col}=1.8\text{mm}$
- $d_{col}=1.2\text{mm}$
Figure 6:

\[ \frac{t_0}{N^2} (s) \]