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Experimental evaluation of chromatographic performance of capillary and microfluidic columns with linear or curved channels

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ABSTRACT

We prepared 0.3 or 0.15 mm i.d. columns from both fused silica capillaries and planar titanium wafers with machined grooves. Both types of devices were packed with sub-two micron C18 sorbent. Chromatographic efficiency and peak capacity were tested using LC instruments with low extra column dispersion (300 nL^2 or 30 nL^2 for 0.3 or 0.15 mm i.d. columns, respectively). Micro column testing in gradient mode was less affected by extra column (pre-column) dispersion. To exploit this feature we developed a method for estimation of column efficiency from gradient analysis using the theoretical relationship $(P_c - 1) = N^{0.5} \times$ const. The validity of this relationship was experimentally verified using 2.1 mm i.d. and 0.3 mm i.d. columns.

The $(P_c - 1)$ versus N relationship was experimentally determined with straight columns, which in turn was employed for the estimation of microfluidic column efficiency. Microfluidic devices with serpentine channels exhibited lower isocratic efficiency than straight capillary columns, but the loss of peak capacity was less significant. The loss chromatographic efficiency due to zone dispersion in serpentine microfluidic channels was more apparent for 0.3 than 0.15 mm i.d. devices. Gradient performance of 0.15 × 100 mm microfluidic columns was comparable to state-of-the-art 2.1 × 100 mm columns packed with the same sorbent.

1. Introduction

Capillary (cLC) or microfluidic (μLC) liquid chromatography columns are used for high sensitivity liquid chromatography mass spectrometry (LC MS) analyses [1–7]. Some users prepare capillary columns in-house while others rely on commercially available products. In either case, having methods for the determination of column quality is critical.

Column performance is traditionally expressed as the number of theoretical plates obtained under isocratic elution conditions [8]. However, the experimentally measured column efficiency (N) is adversely affected by the contribution of extra-column (system) dispersion [9–14]. For separations performed on capillary LC columns of small internal diameter (i.d.), the contribution of the LC system to overall peak dispersion is significant, in many cases precluding a direct measurement of true (intrinsic) column efficiency N_{int}. Extreme precaution must be taken to reduce the LC system dispersion, including minimization of tubing lengths and i.d., developing zero volume connections, optimization of the injector [15], and detection with near-zero dispersion [15–17]. "Pinch" injection (where the sample loop is inserted into the flow path for only a brief moment) [15,18,19], as well as on-column detection (zones eluting from column are detected directly at outlet frit) have been shown to reduce extra column dispersion [15–17].

For cLC column testing, the tubing-derived dispersion must be considered carefully. Recent publications by Grinias et al. [20] and Gritti et al. [9] illustrated that within the practical range of cLC flow rates, the connecting tubing dispersion can be predicted from the Aris-Taylor equation or its modified version. Grinias et al. [20] show that the injector and tubing connections are dominant sources of dispersion in cLC. The importance of low-volume connections in maintaining high performance separations on cLC columns was demonstrated by Franklin [21].

Recently, Gritti et al. [9] investigated the contribution of 2.1 mm i.d. column hardware to band dispersion in UHPLC and estimated it to be between 0.1-0.7 μL^2 for well-designed frits. While this dispersion level is small, it does reduce the achievable efficiency for analytes with low retention, in particular when using short columns packed with sub two-micron sorbent. To our knowledge, the contribution of column hardware to system dispersion in cLC columns has not been systematically studied yet [22], but it is likely to be more significant than in case of 2.1 or 4.6 mm columns. As a conse-

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Table 1
Computational analysis of the impact of LC system extra column variance on observable column efficiency. Calculated for 100 mm long columns with intrinsic efficiencies of 25,000 theoretical plates.

Column i.d.[a] (mm)	Peak variance[b] k = 1.7	System variance (extra column)[c]	Observable efficiency =[d]	System variance to observe 95% of N[e]
2.1	10.65 μL^2	1.5 μL^2	21913	1.18 μL^2
1	0.55 μL^2	1.5 μL^2	6686	0.061 μL^2
0.5	34236 nL^2	300 nL^2	24783	3804 nL^2
0.3	4435 nL^2	300 nL^2	23416	493 nL^2
0.15	277 nL^2	30 nL^2	22559	31 nL^2

[a] Effective column i.d. value. For rectangular chromatographic channels the cross section area corresponds to the listed circular channel i.d.
[b] Peak volume variance σ_v^2 was calculated for a peak with retention factor $k = 1.7$, $t_0 = 0.65$, and flow rate $F = 0.294$ mL/min for 2.1 mm i.d. column. For smaller column i.d. the flow rate was scaled proportionally.
[c] Estimated system variance of ACQUITY I-class system was approximately 1.5 μL^2; cLC systems 1 and 2 used in this study have variance ~300 nL^2 and ~30 nL^2, respectively.
[d] Calculated plate count observable on LC system with given extra-column variance.
[e] Calculated value of extra-column system variance needed to observe 95% of intrinsic column efficiency (0.95 × 25000 = 23750 theoretical plates).

quence, the experimentally observed efficiency N_{obs} of cLC column may be lower than the intrinsic column efficiency N_{int}.

The impact of system dispersion on observed efficiency is illustrated in Table 1 for 100 mm long columns of various internal diameters; the intrinsic efficiency is assumed to be $N_{int} = 25000$. As shown in Table 1, the extra column dispersion must be below 493 nL^2 for 0.3 mm i.d. columns, and below 31 nL^2 for 0.15 mm i.d. columns to experimentally observe an efficiency value that is greater than 95% of N_{int}. However, reducing cLC system dispersion to such levels is difficult. For example, 1 m of 25 μm i.d. capillary tubing generates a variance of 80 nL^2; the same length of 20 μm i.d. capillary gives a variance of 33 nL^2 (calculated from Aris-Taylor equation [23] for diffusion coefficient 1×10^{-5} cm^2/s, and flow rate 1.5 μL/min).

Gradient elution mode has been also employed to measure a column's quality [24–26]. Serving as a surrogate to column efficiency, peak width w or peak capacity P_c becomes the primary indicators of column performance. The advantage of this approach is that well retained analytes are focused on the column inlet at the beginning of analysis, drastically reducing the contribution of sample volume, frits, and pre-column tubing to dispersion [18,19,27]. Some band compression also occurs during gradient elution [28,29]. However, column performance characterization in gradient mode is a less generic approach, since the peak width (peak capacity) also depends on the gradient slope [25,30].

To this end, Snyder and Dolan [31] proposed a model that can be used to measure the column efficiency in gradient mode (Eq. (1)). When including the gradient compression factor, it is possible to estimate the column efficiency with even better accuracy, albeit with the additional need to determine this factor. Neue et al. evaluated an alternative approach for the estimation of column efficiency for proteins [32]. Both methods are potentially useful to measure the performance of cLC columns in gradient mode.

The goal of this work is to investigate the performance of cLC and μLC columns. To accomplish this objective, we investigated a theoretical relationship between column efficiency and peak capacity, and developed a method for column efficiency estimation using gradient analysis of common small molecule analytes without the need to experimentally determine multiple chromatographic parameters.

2. Theory

Snyder and Dolan proposed a relationship between peak width w at gradient elution and column efficiency N [31]. Assuming that the injected sample volume is negligible compared to column volume, the temporal peak width w in linear gradient LC can be calculated from Eq. (1), where D is the gradient compression factor [28,29,31,33], t_0 is elution time of an unretained peak, N is column plate count (efficiency), and k_e is the analyte retention factor at point of elution. This relationship is essentially the same as for isocratic elution, where $D = 1$ and k_e is equal to isocratic retention factor k.

$$w = D\frac{4}{\sqrt{N}}t_0\,(1 + k_e) \tag{1}$$

When rearranged, the relationship can be used to calculate column efficiency from the gradient peak width (measured at 13.4% of peak height; $w = 4\sigma$), assuming that the column efficiency during the gradient elution is constant (Eq. (2)).

$$N = D^2\frac{16}{w^2}t_0^2(1 + k_e)^2 \tag{2}$$

This approach for column plate count measurement in gradient is rarely used in practice, because the gradient compression factor D, and analyte retention factor k_e are not readily known. These parameters have to be estimated experimentally or calculated from gradient theory [8,18,19,28,34].

Neue et al. proposed another gradient method for column efficiency estimation (Eqs. (3)–(5)) assuming linear solvent strength model (LSS), no compression ($D = 1$), and an ideal non-retained gradient [32]. The method was applied to proteins, for which the traditional isocratic measurement of column efficiency is difficult [32]. The symbol σ_t is the peak standard deviation (obtained from peak width in time units measured at 13.4% peak height divided by four), G is the generalized gradient slope defined by Eq. (4), Δc is the difference in the solvent composition over the gradient, t_g represents gradient time, B is the slope of the linear relationship of log k versus solvent composition, and $s = \Delta c \cdot t_0/t_g$ is the gradient slope.

$$N = \frac{t_0^2}{\sigma_t^2}\left(\frac{1}{G} + 1\right)^2 \tag{3}$$

$$G = B \cdot \Delta c\frac{t_0}{t_g} = B \cdot s \tag{4}$$

$$\sigma_t = \frac{t_0}{\sqrt{N}} \cdot \frac{1}{B \cdot s} + \frac{t_0}{\sqrt{N}} \tag{5}$$

The Neue approach is applicable to both large and small molecules, but it neglects the effects of gradient compression [32]. The advantage of the Neue approach is that the B factor can be obtained using several experiments at various gradient slope s by dividing the intercept $\left(\frac{t_0}{\sqrt{N}}\right)$ by slope $\left(\frac{t_0}{\sqrt{N}} \cdot \frac{1}{B}\right)$ of Eq. (5). The B factor is in turn used to obtain a generalized gradient slope G (from Eq. (5)), and column efficiency N (Eq. (3)) [32]. The accuracy of the Neue approach has not been verified by independent reports. Overall, both gradient methods assume the validity of LSS model, which is known to have limitations [18,19].

Here we present an alternative approach for column plate count estimation using a gradient elution method. The proposed method