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Approximate and Exact Equations for Peak Capacity in Isocratic HPLC

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Abstract

In this Letter we examine an equation that had been published in *Analytical Chemistry* in a paper entitled, “Comprehensive Study on the Optimization of Online Two-Dimensional Liquid Chromatographic Systems Considering Losses in Theoretical Peak Capacity in First- and Second-Dimensions: A Pareto-Optimality Approach” by Vivo-Truyols, G., van der Wal, S.J., and Schoenmakers, P. J. *Anal. Chem.* 2010, 82, 8525-8536. In that paper the authors considered, among many issues, the effects of extra-column and column broadening on isocratic peak capacity. They developed an equation to cover all possible conditions, and offered a derivation based on two Taylor-series expansions and a regression. We have found an exact equation that covers all conditions, and have compared the results using our equation to the results using their approximation in predicting ratios of peak widths. Their approximation works well, as we show, but we wish to offer the exact equation which is simpler in form than the approximate solution.

Letter

Vivo-Truyols, van der Wal, and Schoenmakers contributed an article to this Journal (1) in which they studied the loss of theoretical peak capacity in gradient and isocratic online LC X LC separations. Along with several other issues, the authors considered the contribution of extra-column peak broadening to the normal column broadening effects long-noted by those who study HPLC.

As a part of their discussion the authors derived an approximate but nevertheless formidable equation relating isocratic peak capacity to plate count (N), resolution factor (R_s), retention times of first and final peaks ($t_{R,1}$ and $t_{R,n}$), broadening coefficient (d^2), and injection or sampling time (t),

$$n_{iso} = 1 + \frac{\sqrt{N}}{4R_s} \frac{\sqrt{\mu^2 + \frac{1}{d^2}}}{\mu} \ln \left(\frac{t_{R,n} + \frac{t\sqrt{N}}{d^2\mu}}{t + \frac{t\sqrt{N}}{d^2\mu}} \right) \quad (1)$$

where

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$$\mu = \frac{t_{R,1} + t_{R,n}}{2t\sqrt{N}} \quad (2)$$

The derivation of equation (1) (eq 16 in ref 1) was presented in a supplemental information addendum. There the authors invoked a recursion and two Taylor series expansions.

We have found an exact solution that depends on the analytical solution to an integral rather than on the first term of a series expansion. The detailed derivation is attached as supplemental information. The equation is

$$n = 1 + \frac{\sqrt{N}}{4R_s} \left[\sinh^{-1} \left(\frac{t_{R,n}}{\sigma_{ex}\sqrt{N}} \right) - \sinh^{-1} \left(\frac{t_{R,1}}{\sigma_{ex}\sqrt{N}} \right) \right] \quad (3)$$

In which $\sinh^{-1}$ is the arc hyperbolic sine, and σ_{ex} is the measure of peak width due to extra-column broadening, which is equal to t/d . As shown in our supplement and in the nearby figure, in the limit as σ_{ex} approaches zero, this equation becomes identical to the equation derived by Grushka for isocratic peak capacity (2). Interestingly, when σ_{ex} is totally dominant over σ_{col} (column broadening) this equation becomes identical in form to the equation for peak capacity in gradient chromatography assuming a constant peak width (2). Equation (3) predicts observed behavior in the extremes of strong as well as negligible extra-column broadening.

A comparison of the exact and Vivo-Truyols' approximate equations is shown in Figure 1. The vertical axis shows two sets of ratios. The first is the ratio of theoretical peak capacities calculated with Eq.(1) to those calculated without extra column broadening (using Eq.(3) in ref 2). The second is a similar ratio calculated using the exact equation, Eq.(3). The horizontal axis is the ratio of extra-column standard deviation (σ_{ex}) to column standard deviation (σ_{col}). When the ratios σ_{ex}/σ_{col} are extreme, both the exact and approximate equations agree completely. However, for ratios in the midrange (0-1), differences of about 2.5% are found. The assumptions in these calculations are that $N = 10000$ plates, t (sampling time) varies from 1 ms to 40 s, the first peak retention time is 120 s, and the last peak retention time is 600 s, and $R_s = 1.0$.

Conclusions

The agreement between the exact solution and the approximation is really quite good. One can have considerable confidence in the quantitative conclusions of Vivo-Truyols *et al.* even under conditions where the extra-column broadening contributes more to the peak width than does the inherent column broadening. While there is little difference in the conclusions drawn about the quantitative effects of extra-column broadening on theoretical peak capacity using the approximate equation, the simplicity and exactness of the exact equation compared to eq. 1 and 2 recommend its use.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Vivo-Truyols G, van der Wal Sj, Schoenmakers PJ. *Anal Chem.* 2010; 82:8525–8536. [PubMed: 20863086]
2. Grushka E. *Anal Chem.* 1970; 42(11):1142–1147.

Figure 1.
Theoretical peak capacity ratios as a function of the ratio of extra-column to column broadening for the exact and approximate equations.