Waters
Lab Highlights
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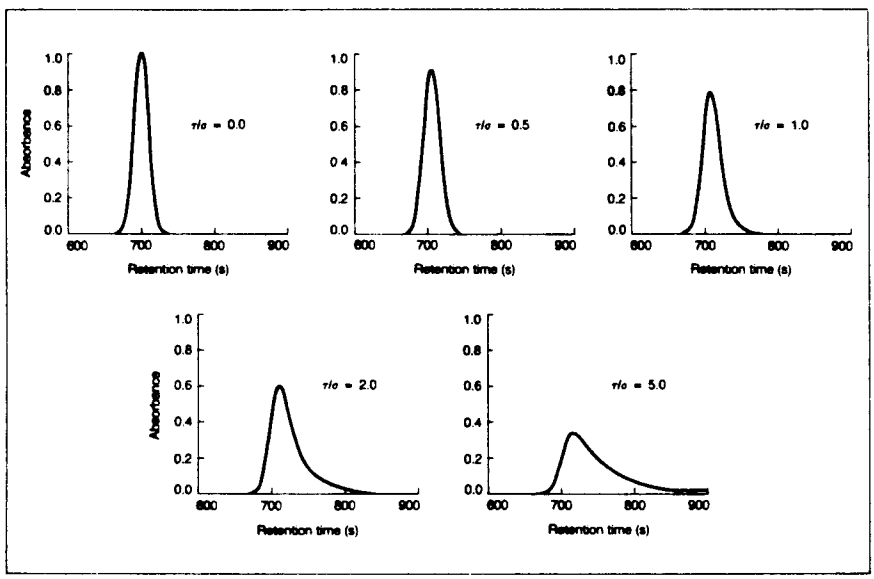
COLUMN EFFICIENCY MEASUREMENT

The proliferation of LC column manufacturers in recent years has resulted in considerable confusion over performance specifications for high-efficiency columns. This is due in part to the fact that each column manufacturer chooses its own procedure for testing, measuring and reporting column efficiency values. The customer is left with the difficult task of making sense of the various measures of efficiency which are reported for commercially available LC columns.

A recent publication (1) from the Applications Development Group at Waters may help to eliminate some of the confusion about column efficiency (i.e. plate count) measurements. This paper provides a concise overview of the topic, focusing specifically on the alternatives for measurement and calculation of column efficiency values. In addition, a critical comparison of nine different calculation methods is reported. This comparison is based on the computerized evaluation of a series of synthetic tailed peaks (see Figure 1).

FIGURE 1



Exponentially modified Gaussian peak profiles for τ/σ = 0.0, 0.5, 1.0, 2.0, and 5.0; constant mean of 700 s and σ_G of 10.00 s, with τ increasing

The results of the comparison of calculation methods are of particular interest to any purchaser of LC columns. The various alternatives for calculating column efficiency differ significantly in their accuracy when faced with tailing chromatographic peaks. Figure 2 demonstrates the differences among nine calculation methods in a plot of column efficiency (plates/column) vs. the tau/sigma tailing parameter. As tau/sigma increases from 0.0 to 2.0, the actual column efficiency is degraded, as indicated by the peak shapes in Figure 1.

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While all of the calculation methods show a decrease in the number of plates per column as tau/sigma increases, some methods are considerably more accurate than others. In Figure 2, the true plate count is given by the moments method, which traces out the lowest curve in the figure. The least accurate efficiency measurement is given by the inflection, or two-sigma method, which gives the upper curve of Figure 2. The five-sigma method used by Waters gives excellent accuracy, particularly in comparison to the popular half-peak-height method.

FIGURE 2

Trend in column efficiencies computer calculated by nine methods, as a function of increasing peak asymmetry for profiles of the type shown in Figure 1. Key to methods: 2S = two sigma, 3S = three sigma, 4S = four sigma, 5S = five sigma, T = tangent, H = half peak height, AH = area/height, M = moment, and A = asymmetry based.

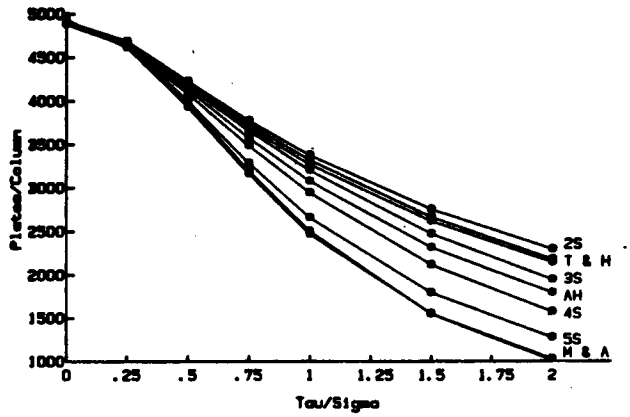

Table I provides a quantitative comparison of the nine calculation alternatives, by comparing the efficiency values for a moderately tailed peak (tau/sigma = 2.0). This degree of tailing would be found in many practical applications of LC, although the test mixtures used for most column efficiency tests will exhibit relatively less tailing. The inflection (two-sigma) method gives an answer which is 127% above the actual plate count, while the five-sigma value is inflated by only 26%. The half-height method gives an efficiency value which is 77% too high. Note that for more symmetric peaks, the trend in Table 1 would still hold, but the percent inaccuracy would be reduced.

Table 2 indicates the calculation methods which are used by some LC column suppliers. Since there are presently over 65 suppliers, Table 2 is not intended to be an exhaustive list. The important point to note is the tendency for LC column suppliers to choose a calculation method which will give an inaccurate efficiency value when applied to tailed chromatographic peaks. Waters chooses to apply the stringent five-sigma method, thus insuring a more accurate determination of column efficiency.

TABLE I. Comparison of Efficiency Values for a Synthetic Chromatogram by Nine Calculation Methods.

	N(plates/column)	Inaccuracy
Inflection (Two-Sigma)	2291	127%
Half-Peak-Height	2175	115%
Tangent	2138	112%
Height/Area Ratio	1790	77%
Four-Sigma	1569	55%
Five-Sigma	1276	26%
Asymmetry-Based	1028	2%
Moments	1010	0%
Actual	1010	-

TABLE II. Calculation Methods for Column Efficiency Used by LC Column Suppliers

Company	Calculation Method for Bonded-Phase Columns [a]
Beckman	Half Peak Height
Du Pont	Height/Area Ratio
IBM	Half Peak Height
Merck	Half Peak Height
Perkin-Elmer	Half Peak Height
Supelco	Half Peak Height
Varian	Half Peak Height
Waters	Five Sigma
Whatman	Inflection (Two Sigma)

[a] Source: Manufacturer's literature or personal communication

1. B. A. Bidlingmeyer and F. V. Warren, Anal. Chem., 56 (1984) 1583A. (Available as Document #T88/81508 from the Waters Literature Center.)