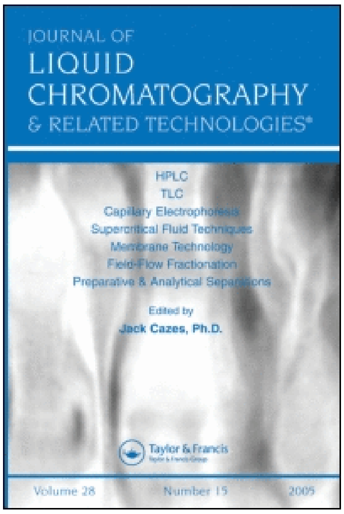

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On the Use of the Knox Equation. II. the Efficiency Measurement Problem

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ON THE USE OF THE KNOX EQUATION. II. THE EFFICIENCY MEASUREMENT PROBLEM

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ABSTRACT

The Knox equation, which relates the reduced plate height, h, to the reduced linear velocity, $\underline{v}$, has a great importance in column and stationary phase testing. Significant A, B, and C terms of the Knox equation cannot be obtain with erroneous plate heights, h. Four methods for efficiency determination, the inflection (0.6H) method, the height/area method, an asymmetry based (0.1H) method and the moment method, were critically compared using 200 real chromatograms. Efficiency obtained using Gaussian assuming methods were highly overestimated, while the asymmetry based method gave quite acceptable results. Using the exponentially modified Gaussian model, it is demonstrated that the **b/a** ratio (the sum **b** + **a** is the peak-width at 10% of the peak height) is not an empirical figure of merit but can be related to a "peak skew" expression.

The A, B, and C terms of the Knox equation:

$$h = A\,\underline{v}^{1/3} + B/\underline{v} + C\underline{v} \qquad \text{Eq. 1}$$

have a great practical and theoretical importance. The A term is used to assess the goodness of a column packing. The B term is related to solute longitudinal diffusion. The C term depends on the solute mass-transfer between phases (1-3). Although other general plate height equations were proposed by Giddings (4), Snyder (5), Huber (6) or Horvath (7), the Knox equation is the most widely used for column characterization.

In the first part of this study, the fit problem was exposed (8). It was shown that great care must be taken when using conventional fitting methods. The lowest confidence interval obtainable for the fitted A, B, and C terms of the Knox equation was in the 20% range.

It may seem trivial that significant A, B, and C terms cannot be obtain with erroneous plate height measurements. However, commonly used methods for plate count determination produce overestimated plate numbers and, consequently, biased Knox terms. The aim of the second part of this study of the Knox equation was to show that the way the efficiency is measured is great concern of the significance of the A, B, and C terms obtained.

Determination of column efficiency can be done using more than ten different methods. Among which there are the width at half-height method, the tangent method, the inflection method (0.6H method), the three, four, or five sigma methods, the peak-height over area method, asymmetry based methods and the moment method (9). The last method is the only one that makes no assumption about the peak shape, that is why the moment method was given to be the only absolute method (10). Several authors compared plate counts obtained with various methods (9, 11). The moment method was confirmed to have the highest exactitude. However, because the moment method imposes the use of a computer, the comparison of methods was done with computer-synthesized peaks of known efficiency (9, 11). In this work, we propose to compare four plate-determination methods computing the efficiency of more than 200 real chromatographic peaks obtained for other purposes with a potpourri of stationary phase-mobile phase combinations on optimized systems (12) or non-optimized systems with fronting and tailing peaks (in micellar liquid chromatography) (13, 14).

1- Theoretical part

Column Efficiency measurements

The four methods chosen were (i) the popular 0.6H method:

$$N_{0.6H} = 4(t_r/W_{0.6H})^2 \qquad \text{Eq. 2}$$

in which $N_{0.6H}$ is the plate number, t_r is the retention time and $W_{0.6H}$ is the peak width at 60% of the peak height in time unit; (ii) the peak-height/area (H/A) method:

$$N_{H/A} = 2\pi\ (Ht_r/A)^2 \qquad \text{Eq. 3}$$

(iii) the asymmetry based method derived by Foley and Dorsey (15) which uses the peak width at 10% of the peak height ($W_{0.1H}$) and the asymmetry ratio **b/a** (with **a** and **b** referred to the peak maximum and $\mathbf{a} + \mathbf{b} = W_{0.1H}$):

$$N_{0.1H} = [41.7\ (t_r/W_{0.1H})^2]\ /\ [\mathbf{b/a} + 1.25] \qquad \text{Eq. 4}$$

and (iv) the moment method:

$$N_{moment} = M_1^2\ /\ M_2 \qquad \text{Eq. 5}$$

in which M_i is the ith moment. M_0 is the peak area and is defined as:

$$M_0 = \quad C(t)\ dt$$

with C(t) the detector signal. The first reduced moment corresponds to the peak retention time:

$$M_1 = \quad [t\ C(t)/M_0]\ dt$$

M_2 is the second central reduced moment and corresponds to the peak variance (10). Moment calculation cannot be done by hand; an analogic-digital converter is needed to digitize the chromatographic peak into at least 50 points which can be used by a computer for moment calculation.

Exponentially modified Gaussian peak

The efficiency measurement problem originates in the Gaussian shape peak approximation. Giddings has pointed out that, without any extra-column band-broadening, the simplest conceivable retention mechanism (single step sorption-desorption) does not lead to a Gaussian profile, but to a form containing a Bessel function and an exponential term (4, 10).

The Gaussian model, convoluted by an exponential function, is a much closer representation of real peaks (16). The exponentially modified Gaussian (EMG) equation is:

$$f(t) = [S/(\tau\sigma\sqrt{2\pi})] \int \exp\{-[t-t_G-t']/(2\sigma^2)\} \exp(-t'/\tau)\, dt' \qquad \text{Eq.6}$$

in which S is the peak surface area, σ is the variance of the Gaussian factor, t_G is the retention time of the Gaussian factor, τ is the time constant of the exponential modifier term and t' is a dummy variable of integration (17). With the EMG equation, the moments can be simply expressed as:

$$M_1 = t_G + \tau \qquad \text{Eq. 7}$$

$$M_2 = \sigma^2 + \tau^2 \qquad \text{Eq. 8}$$

$$M_3 = 2\tau^3 \qquad \text{Eq. 9}$$

The magnitude of the time constant τ corresponds to the Gaussian profile departure. With a τ/σ ratio lower than 0.5, a peak is almost gaussian. Non Gaussian peaks often present a tail which is evaluated with the peak "skew".

Peak skew

The third reduced central moment is used in "Skew" calculation:

$$\text{"Skew"} = M_3/M_2^{3/2} = 2\tau^3 / (\sigma^2 + \tau^2)^{3/2} \qquad \text{Eq. 10}$$

In this work, we chose not to calculate third or higher moments because these moments cannot be computed with sufficient accuracy for real peaks. This is due to computer data truncating at elevated retention times and also to high noise-sensitivity. Although the only statistically exact definition of the skew is Eq. 10, we chose to use a "peak skew" defined by Rocca et al. (18) as:

$$\text{"peak skew"} = (M_1 - t_r) / M_2^{1/2} \qquad \text{Eq. 11}$$

The "peak skew" expressed by Eq. 11 is related to the exact skew (Eq. 10) by:

$$(M_1 - t_r) / M_2^{1/2} = [\tau - (t_r - t_G)] / (\sigma^2 + \tau^2)^{1/2} = (\text{Skew}/2)^{1/3} - (t_r - t_G)/(\sigma^2 + \tau^2)^{1/2}$$

When the peak Gaussian departure is high, the term $(t_r - t_G)/(\sigma^2 + \tau^2)^{1/2}$ becomes low and the "peak skew" value of Eq. 11 becomes close to the cubic root of the half of the genuine "Skew" (Eq. 10). Grushka demonstrated that the maximum value of the "Skew" was 2 (17), then, the "peak skew" value (Eq. 11) is always lower than 1.

2- Comparison of four plate count methods

The moment method was demonstrated to be the most accurate method. So, the three other efficiency measurement methods (0.6H, H/A, and 0.1H) were compared to the moment method assumed to produce the exact plate count of the real peaks. The criterion chosen to quantify the accuracy of a plate number, N_{method}, was the relative deviation (in percent) referred to the moment plate number, N_{moment}:

$$\text{Deviation \%} = [100*(N_{method} - N_{moment})] / N_{moment}$$

This deviation was compared for peaks with similar skew. Figures 1-3 are tridimensional (3D) plots of the deviation (z axis) for a method versus the moment plate count (x axis) and the peak

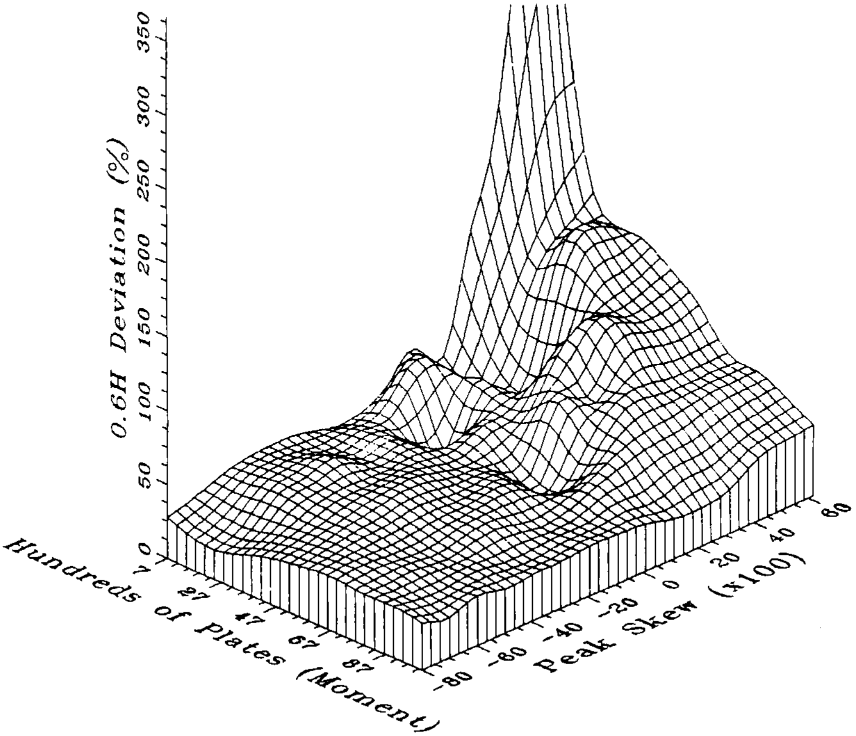

Figure 1: The significance of efficiency measurements using the 0.6H method. Tridimensional plot of the inflection (0.6H) method deviation versus the exact efficiency, obtained by the moment method (X axis) and the peak skew (Y axis) obtained using 213 actual peaks. Negative peak skew corresponded to peak fronting.

skew (y axis). Peaks with fronting were treated as peaks with a negative tailing ($\tau<0$).

Inflection (0.6H) method

Figure 1 presents the 3D-plot corresponding to the 0.6H method. In all cases, the plate count obtained by this method was overestimated by at least 30% with respect to the moment method value. The peak skew effect is obvious. The divergence increases as the peak skew increases, becoming dramatic for low efficiency columns. With a peak skew of 0.6, the deviation was 70% (overestimation) for peaks with 9000 actual plates (15300 plates, 0.6H method), it skyrocketed to 450% for peaks bearing only 700 actual plates (3200 plates, 0.6H method).

Height/area method

For the H/A method, Figure 2 presents a 3D-diagram plotted the same way as Figure 1. The general trend is similar, but the H/A method produced better efficiency values at low peak skew and/or with good columns. Whatever peak skew, the H/A deviation was lower than 25% with 9000 plate or better columns. With 700 plate columns, the deviation could reach 280% with a peak skew of 0.6. For all peaks, the H/A method gave plate counts closer to the actual value than the 0.6H method. Most often the H/A deviation was two times lower than the corresponding 0.6H deviation.

Asymmetry based (0.1H) method

The asymmetry based method was not supposed to be used with fronting peaks (15). The use of Eq. 4 with such peaks produced **b/a** ratio lower than unity and, consequently, plate counts higher than purely Gaussian peaks. To extend the use of the Dorsey's equation (Eq. 4), we chose arbitrarily to put in Eq. 4 the ratio **a/b** instead of **b/a** in the case of fronting peaks. This produced the Figure 3 plot with slightly underestimated plate counts (-20%) for severely fronting peaks (peak skew lower than -0.6) and low efficiency (plate number lower than 4000).

In general, the 0.1 method gave exact plate counts (deviation lower than 6% with respect to the moment method) for most experimental peaks studied. The highest deviation was 80% for very bad columns (700 plates) and severely tailing peaks (peak skew 0.6). Figures 1, 2, and 3 clearly show that the 0.1H method is better than the two other methods. This method was previously given as the best manual method for plate count evaluation (9). the present evaluation with real chromatographic peaks is in full agreement with evaluations performed with computer synthesized peaks (9, 11).

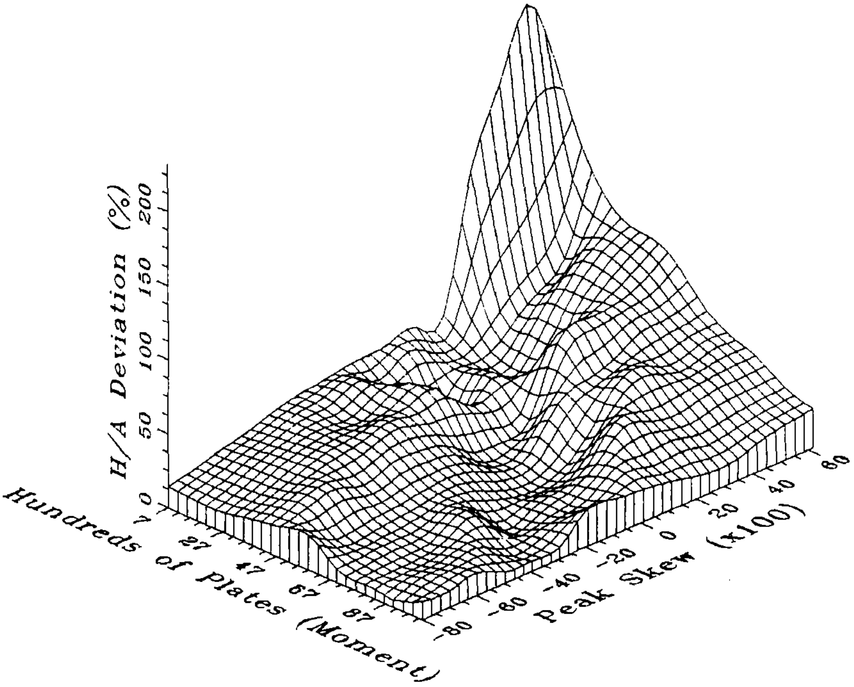

Figure 2: The significance of efficiency measurements using the peak-height over area method. See Figure 1 caption.

As an interesting side-result, as shown by Figure 4, we found that the peak skew (Eq. 11) could be related to the **b/a** ratio by:

$$\mathbf{b/a} = \exp\left[(3/2)*\text{"peak skew"}\right] = \exp\left[(3/2)(M_1-t_r)/M_2^{1/2}\right] \quad \text{Eq. 12}$$

Eq. 12 is valid for negative peak skew. Given the variety of mobile and stationary phases, solutes and column lengths, the probability for this relationship to be serendipitous is low. If Eq. 12 is confirmed, it links the **b/a** ratio to well-established chromatographic figure of merit, retention time, mass center and peak variance and the **b/a** ratio would no longer be an empirical figure of merit. For example, assuming Eq. 12 is exact, it is possible to derive mathematical relations corresponding to the graphical method proposed by Barber and Carr (19). This is exposed in Annex.

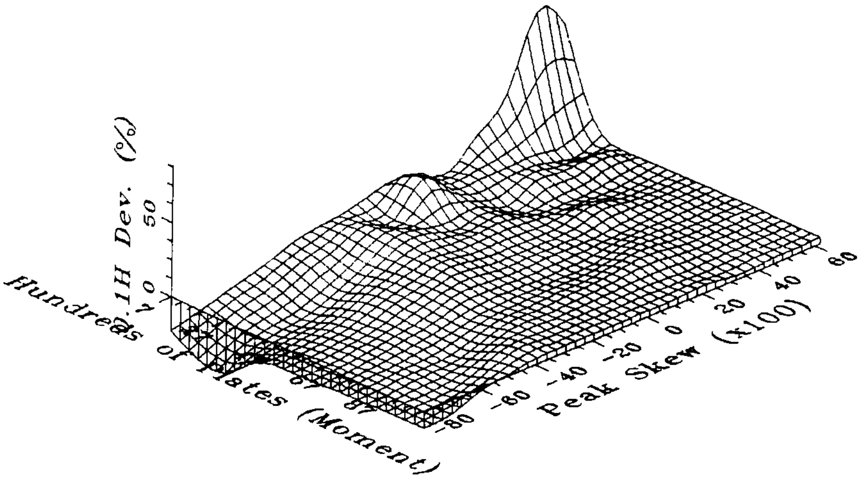

Figure 3: The significance of efficiency measurements using the asymmetry based (0.1H) method. See figure 1 caption. For negative peak skew (peak fronting), the ratio **a/b** was used in Eq. 4 instead od **b/a**.

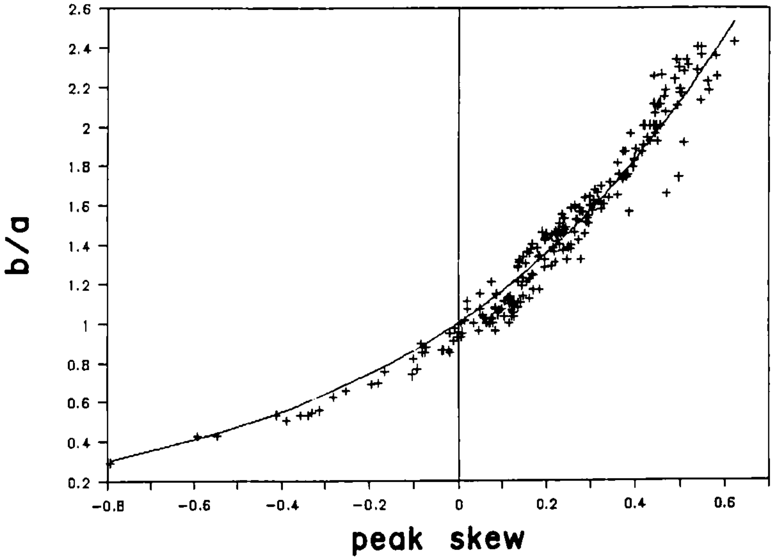

Figure 4: Plot of the **b/a** ratio versus the experimental peak skew for 213 actual peaks.
Full line: curve of the equation: **b/a** = exp(1.5 x skew).

3- Efficiency and Knox plots

Obviously, different sets of plate heights, h, versus reduced velocities, $\underline{v}$, produce different A, B, and C terms. In a recent work using the B parameter of the Knox equation, we found B values leading to obstruction factor, γ_m, higher than unity (12). A γ_m factor higher than 1 is theoretically impossible; that would mean the solute diffusion in the mobile phase inside the porous stationary phase is easier and faster than the solute diffusion in the bulk mobile phase. We thought that the main reason explaining why γ_m factors higher than unity were not reported previously was the efficiency measurement method. In most previous work, band broadening were estimated assuming Gaussian peaks, this led to underestimated B values (12).

Figure 5 displays the different fits obtained with the same set of chromatograms (solute: benzene, k'=0.541), but the efficiency was measured using the four different methods described above. Table I lists the corresponding A, B, and C terms and the corresponding error of fit. One can see that the moment method and the asymmetry based method (0.1H method) gave close results that was the reason why the 0.1H fits was not displayed on Figures 5 and 6: they were very close to the moment method curves. Figure 6 corresponds to the solute propylbenzene (k'=2.43) on the same column.

Table I lists the Knox terms with the confidence limits. A and C terms obtained by fitting Knox plots where h values were obtained using Gaussian assuming methods, can be 50% to 500% off the exact values, underestimating them. The corresponding B values seem to be only 20% to 40% off, due to the fact that the B term depends on very low flow rate experiments. At low flow rates, peak tailing decreased, and almost Gaussian shapes could be found, specially for solutes with elevated k'.

The high sensitivity of fit procedure to low variation in experimental results was illustrated using the point focussed by an arrow (Figure 5). With this point, the least square fit method (see Part I) gave the values 1.50, 4.0, and -0.06, for the A, B, and C terms, respectively. The visual fit method (8) suggested that the fit could be better without that arrowed point. The new fit, without the

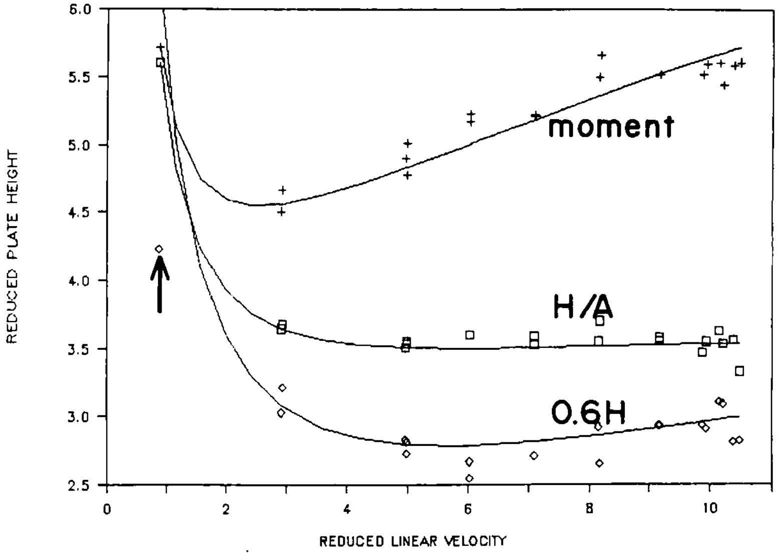

Figure 5: Knox plots obtained with the same 19 benzene peaks but the efficiency was determined with the quoted methods. Mobile phase: methanol-water 80-20% v/v; Column 15 cm x 4 mm i.d., nucleosil C18, 5 ηm. See Table I.

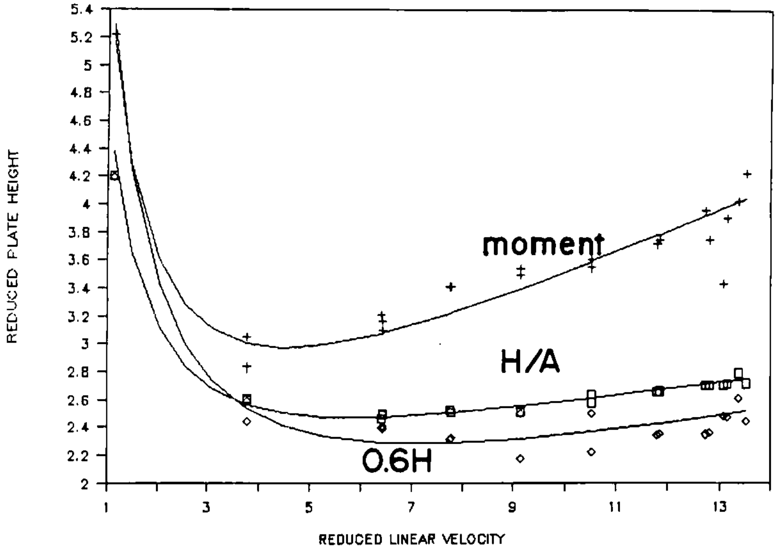

Figure 6: Knox plots obtained with the same 19 propylbenzene peaks but the efficiency was determined with the quoted methods. Mobile phase: methanol-water 80-20% v/v; Column 15 cm x 4 mm i.d., nucleosil C18, 5 ηm. See Table I.

Table I - Parameters of Figures 5 and 6

Benzene, Figure 5

$k' = 0.541,\ D_m = 8.65\times10^{-6}\,cm^2/s$

Efficiency method	A	B	C	Error of fit	Symbol
moment	2.49 ±0.05	4.80 ±0.2	0.0 ±0.02	0.02 ±0.005	+
0.1H	2.47 ±0.15	4.80 ±0.2	-0.01 ±0.03	0.025 ±0.01	
H/A	1.95 ±0.25	5.5 ±0.5	-0.1 ±0.05	0.006 ±0.005	□
0.6H	0.98 ±0.10	7.5 ±0.5	0.04 ±0.02	0.010 ±0.007	◇
0.6H	1.50 ±0.25	4.0 ±0.4	-0.06 ±0.04	0.024 ±0.005	↑

Propylbenzene, Figure 6

$k' = 2.43,\ D_m = 6.5\times10^{-6}\,cm^2/s$

Efficiency method	A	B	C	Error of fit	Symbol
moment	0.81 ±0.20	7.5 ±0.5	0.13 ±0.03	0.028 ±0.004	+
0.1H	0.79 ±0.20	7.8 ±0.6	0.11 ±0.04	0.019 ±0.006	
H/A	0.98 ±0.05	6.0 ±0.2	0.01 ±0.01	0.0008 ±0.0002	□
0.6H	0.75 ±0.20	6.2 ±0.5	0.04 ±0.02	0.016 ±0.006	◇

point, gave 0.98, 7.5, and 0.04 for A, B, and C, respectively. The error of fit was five times lower. A change of only one point induced a variation of -53%, +47%, and +250% on the A, B, and C term, respectively. Although this example was chosen to point out dramatically the fit problem, it illustrates the extreme care that one must take when fitting h vs **v** plots to get the A, B, and C terms of the Knox equation. It depends on what these terms are wanted for. In evaluation of fundamental properties of a new stationary phase or in solute-stationary phase interaction studies, the confidence limits for each Knox parameter must be given. In practical cases, when different columns are routinely compared using the same chromatographic system, the same mobile phase and the same solutes, a plot of h, the reduced plate height, versus F, the flow rate, is sufficient to determine the best column. However, a Gaussian assuming plate height determination will always overestimate the column efficiency and may overlook some important problems causing peak tailing. If there is no computer available to use the moment method for efficiency evaluation, the asymmetry based method (0.1H method) is the best substitute.

References

1- Knox, J.H.; Saleem, M., **J. Chromatogr. Sci.**, 10, 80 (1972).

2- Done, J.N.; Knox, J.H., **J. Chromatogr. Sci.**, 10, 606 (1972).

3- Knox, J.H., **J. Chromatogr. Sci.**, 15, 352 (1977).

4- Giddings, J.C., **Dynamics of Chromatography**, Marcel Dekker, New York, 1965.

5- Snyder, L.R., **J. Chromatogr. Sci.**, 7, 352 (1969).

6- Huber, J.F.K., **J. Chromatogr. Sci.**, 7, 86 (1969).

7- Horváth, C.; Lin, H.J., **J. Chromatogr.**, 149, 43 (1978).

8- Berthod, A., **J. Liq. Chromatogr.**, submitted.

9- Bidlingmeyer, B,A.; Warren, F.V., **Anal. Chem.**, 56, 1583A (1984).

10- Grushka,E.; Myers, M.N.; Schetter, P.D.; Giddings, J.C., **Anal. Chem.**, 41, 889 (1969).

11- Kirkland, J.J.; Yau, W.W.; Stoklosa, H.J.; Dilks, C.H., **J. Chromatogr. Sci.**, 15, 303 (1977).

12- Berthod, A.; Chartier, F.; Rocca, J.L., **J. Chromatogr.**, in press.

13- Berthod, A.; Roussel, A., **J. Chromatogr.**, 449, 349 (1988).

14- Berthod, A.; Girard, I.; Gonnet, C., **Anal. Chem.**, 58, 1362 (1986).

15- Foley, J.P.; Dorsey, J.G., **Anal. Chem.**, 55, 730 (1983).

16- Gladney, H.M.; Dowden, B.F.; Swalen, J.D., **Anal. Chem.**, 41, 883 (1969).

17- Grushka, E., **Anal. Chem.**, 44, 1733 (1972).

18- Rocca, J.L.; Higgins, J.W.; Brownlee, R.G., **J. Chromatogr. Sci.**, 23, 106 (1985).

19- Barber, W.E.; Carr, P.W., **Anal. Chem.**, 53, 1939 (1981).

20- Yau, W.W., **Anal. Chem.**, 49, 395 (1977).

ANNEX

Relation between τ/σ and b/a, the asymmetry ratio

Eq. 11 can be rewritten using Eqs 7 and 8:

$$(M_1 - t_r) / M_2^{1/2} = [\tau/\sigma - \{(t_r - t_G)/\sigma\}] / [1 + (\tau/\sigma)^2]^{1/2} \qquad \text{Eq. A1}$$

Barber and Karr (19) proposed an abacus to obtain graphically $(t_r - t_G)/\sigma$ when τ/σ was known. Using the method of Yau (20), which noticed that the peak maximum, at time t_r, falls on the contour of the Gaussian constituent, one can write:

$$H_{EMG} = [S/(\sigma\sqrt{2\pi})] \exp [(t_r - t_G)^2/2\sigma^2]$$

and

$$|(t_r - t_G)/\sigma| = [2 \text{ Log } \{S/(H_{EMG}\sigma\sqrt{2\pi})\}]^{1/2} = [2 \text{ Log } (H_G/H_{EMG})]^{1/2}$$

in which H_{EMG} and H_G are the EMG peak height and the Gaussian peak height, respectively, S is the peak area. From ref. 20, it can be derived:

Table II - Calculated asymmetry parameter b/a

τ / σ	1	2	4	6	8	10	15	20	40	100
1	1.36	1.94	2.63	3.00	3.25	3.41	3.67	3.83	4.10	4.30
2	1.06	1.36	1.94	2.35	2.63	2.84	3.20	3.41	3.83	4.16
4	1.00	1.06	1.36	1.67	1.94	2.16	2.57	2.84	3.41	3.93
8	1.00	1.00	1.00	1.20	1.36	1.52	1.88	2.16	2.84	3.56
12	1.00	1.00	1.00	1.06	1.14	1.25	1.52	1.78	2.45	3.28
16	1.00	1.00	1.00	1.00	1.06	1.13	1.32	1.52	2.16	3.04
20	1.00	1.00	1.00	1.00	1.01	1.06	1.20	1.36	1.94	2.84
25	1.00	1.00	1.00	1.00	1.00	1.01	1.11	1.23	1.73	2.63
30	1.00	1.00	1.00	1.00	1.00	1.00	1.06	1.15	1.56	2.45
60	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.15	1.77

As the variable is the τ/σ ratio, the **b/a** value for $\tau=60$ and $\sigma=12$ is 2.84 ($\tau/\sigma = 5$). Note that Eq. 4 was given for use in the $1.09 <$ **b/a** < 2.76 range (15) and that the maximum "peak skew" being 1, the maximum theoretical **b/a** ratio is 4.48 (exp 1.5, Eq. 12).

$$H_G / H_{EMG} = 1 + [(\tau/\sigma)^2 / \{1 + \sqrt{2\pi}\tau/\sigma\}]$$

and

$$|(t_r - t_G)/\sigma| = [2 \text{ Log } \{1 + [(\tau/\sigma)^2 / (1 + \sqrt{2\pi}\tau/\sigma)]\}]^{1/2} \qquad \text{Eq. A2}$$

Equation A2 is the mathematical expression corresponding to the abacus of Figure 4 of Reference 19. Combining Eqs 12, A1, and A2, we get:

$$2/3 \text{ Log}(\mathbf{b/a}) = \frac{\tau/\sigma - [2 \text{ Log } \{1 + [(\tau/\sigma)^2 / (1 + \sqrt{2\pi}\tau/\sigma)]\}]^{1/2}}{[1 + (\tau/\sigma)^2]^{1/2}}$$

Table II lists the calculated **b/a** ratio for different τ and σ values.