Journal of Chromatography, 630 (1993) 69–77
Elsevier Science Publishers B.V.. Amsterdam

CHROM. 24 687

Improved computer algorithm for characterizing skewed chromatographic band broadening

II. Results and comparisons

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(First received August 20th, 1992; revised manuscript received October 14th, 1992)

ABSTRACT

A newly developed method using an exponentially modified Gaussian peak shape model produces results that are more precise and less subject to baseline noise than previous methods for characterizing chromatographic band broadening. The method requires only precisely measurable experimental peak parameters: peak retention time, peak height, peak area, and peak centroid (first moment). Accuracy and precision of the new method were compared with other digital approaches by using computer-synthesized peaks and experimental chromatographic data from many HPLC columns. The proposed method offers a reasonable compromise between accuracy, precision, and convenience. A rapid visual estimate of peak skew can be made by inspecting peak shape and referring to a calibration plot involving peak parameters. Peak variance and skew data from this method are also useful for finding column dispersion corrections in size-exclusion chromatography calibrations.

INTRODUCTION

Accurate and precise information from real peaks is needed for many analytical applications in gas and liquid chromatography, and other separation methods. This is often a challenging task that requires sophisticated computational methods involving computers. Since the simple Gaussian model can produce serious errors in finding plate number, peak asymmetry factor, and resolution [1], various other approaches have been proposed. The most accepted and used of these is based on the exponentially modified Gaussian (EMG) model [2–6]. Reviews of EMG uses have been given [7,8]. Empirical equations using EMG models have been proposed for calculating chromatographic figures of merit [9], and the effect of random noise on measurements by the EMG model has been addressed [10].

A new method of extracting band-broadening parameters from noisy and skewed chromatographic peaks recently was proposed [11]. This method also is based on the EMG model, but is more accurate and less susceptible to baseline noise than previously used methods. In this new procedure (the “DuPont method”), only four easily and precisely-determinable peak parameters are measured on an experimental chromatographic peak: (1) peak reten-

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tion time, (2) peak height, (3) peak area, and (4) peak centroid.

To evaluate efficacy, we compared the DuPont method with true moment calculations, the James–Martin method [12], and the Dorsey-Foley method (sometimes called the Foley-Dorsey method) [6]. These different methods provide distinctive features and offer different levels of compromise between accuracy and precision. In the present study, critical comparisons established the limitations, applicabilities, and performance of these methods. In one part, we used computer-synthesized chromatographic peaks containing built-in noise and baseline drift. Peaks created with the EMG model permitted a careful study of peak skew measurements. Other investigated parameters included the effects of random *versus* cyclic baseline noise, and various degrees of baseline drifts. Only results with random baseline noise are given in this paper. Results obtained with cyclic noise and baseline drift did not significantly change results or the conclusions regarding the proposed new DuPont method. An advantage of a computer-simulation study of this type is the complete objectivity that is possible. Also, computer simulation permits the study of a wider range of peak shape differences than is conveniently available from experimental approaches. Identical computer-synthesized data sets were used to test the different peak characterization methods.

The second part of this study involved the use of "real" chromatographic peaks experimentally developed using seventeen columns with a wide range of types and characteristics. With data from these columns, we developed a quantitative comparison of the various peak characterization methods.

THEORY

The new digital DuPont method of characterizing chromatographic peaks [11] requires only precisely measurable peak parameters for the calculation. These consist of peak retention time, t_p; peak height, h_p; peak area, M_0; and, the peak centroid (first moment), M_1. Fig. 1 illustrates the needed parameters. A chromatographic peak can be described as a time distribution of the peak height h (t) at any retention time, t. A real chromatographic peak usually can be reliably described by a Gaussian distribution modified with an exponential function,

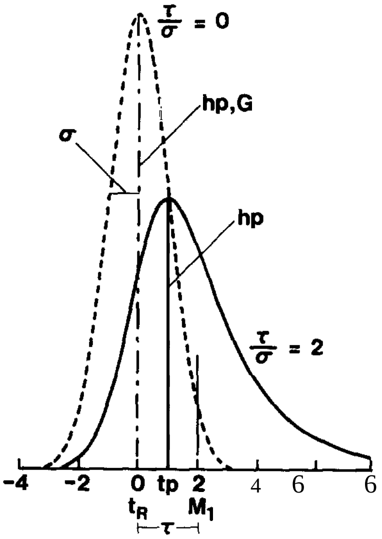

Fig. 1. Peak shape model for exponentially modified Gaussian (EMG). From ref. 10.

where σ is the standard deviation or width at 60.7% of the height of the Gaussian component, τ is the exponential time decay constant, and t_G is the retention time for the Gaussian component of the peak. The EGM peak model can be described as:

$$h\,(t) = \frac{M_0}{\tau\sigma\sqrt{2\pi}} \int_0^\infty \exp\left[-\left(\frac{t - t_G - t'}{\sqrt{2}\,\sigma} \right)^2 - \frac{t'}{\tau} \right] \mathrm{d}t' \quad (1)$$

where t' = the integration retention time variable. The statistical moments of the peak can be mathematically defined, and these statistical moments can be related to the peak shape parameters, as summarized in Table I.

Moment method

For the peak moment method, calculation of plate number, N, skew, and peak variance σ^2 is determined by a point-by-point summation of terms within the integral from the beginning to the end of the peak (eqns. 2-7, Table I). This method assumes no peak shape and can produce the most accurate results. However, it is well-known that moments are quite sensitive to baseline noise. Precision often is too poor for the practical characterization of typical chromatographic peaks.

TABLE I

CHARACTERISTICS OF CHROMATOGRAPHIC PEAKS

Characteristic	Gaussian calculation	Eqn. No.	EMG calculation	Eqn. No.
m_0 (area)	$\int_0^\infty h(t)\,dt$	2		
m_1 (centroid)	$\int_0^\infty \frac{t h(t)\,dt}{m_0}$	3	$t_R + \tau$	
m_2 (variance)	$\int_0^\infty \frac{(t-m_1)^2 h(t)\,dt}{m_0}$	4	$\sigma^2 + \tau^2$	9
m_3	$\int_0^\infty \frac{(t-m_1)^3}{m_0} h(t)\,dt$	5	$2\tau^3$	10
Peak skew	$m_3/m_2^{3/2}$	6	$2(\tau/\sigma)^3/(1+\tau^2/\sigma^2)^{3/2}$	11
Plate number	m_1^2/m_2	7	$(t_R+\tau)^2/(\tau^2+\sigma^2)$	12

James-Martin method

The James-Martin (J-M) method [12] assumes a strict Gaussian peak shape. Plate number is calculated as

$$N = (t_p/\sigma)^2 \qquad (13)$$

or,

$$N = 2\pi (h_p t_p/M_0)^2 \qquad (14)$$

Since a Gaussian peak shape is presumed, no information on peak skew can be obtained with this method.

Dorsey-Foley method

This method (DF-NA) uses the EMG model [6] that was available on the original Nelson Analytical PC software package. The expression for calculating plate number is:

$$N_{SYS} = [41.7(t_p/W_{0.1})^2]/[(B/A) + 1.25] \qquad (15)$$

where N_{SYS} = plate number of a given (asymmetric) chromatographic system; $W_{0.1}$ = peak width at 10% of the peak height; B/A is the peak asymmetry factor at $W_{0.1}$. An upgrade version of this method (DF) was later available with improved peak detection and baseline logic.

DuPont method

The relationships for the DuPont method also utilize the EMG model. The theory and background for this method is detailed in a companion publication [11] and will not be repeated here. In this previous publication, a fully digital method is described, as well as a simplified computer algorithm with a graphical illustration.

EXPERIMENTAL

Computer-simulated peaks were generated with a VAX 3 100 computer (Digital Equipment Corporation, Maynard, MA, USA) using in-house developed software. Fig. 2 illustrates the conformation of simulated peaks with various levels of imposed noise. Synthesized peaks of this type with various level of plate number and peak skew (with and without noise) were analyzed with the various peak characterization methods described above. For the simulation studies, moment, James-Martin, and DuPont calculations were made with in-house-developed software on a VAX Model 3100 computer. Dorsey-Foley calculations for the simulation study were carried out with commercial software

(Nelson Analytical, Cupertino, CA, USA) installed on the same computer. Dorsey-Foley measurements on columns were made with two versions of PC-based software (Nelson Analytical). Baseline "white" noise was produced with a random-noise generator for all methods. A two-points per second sampling rate was used to collect all data in this study.

Test chromatographic separations were made with a Model 8800 liquid chromatograph (DuPont Instruments Division, Wilmington, DE, USA), using 25 x 0.46 cm I.D. Zorbax-ODS, -C$_8$, and Zorbax-PSM-60S columns also from DuPont. The chromatographic test mixture contained uracil (unretained), phenol, 4-chloronitrobenzene, and toluene, shown in Fig. 3 with the mobile phases used for each column. This study used a total of seventeen commercial and experimental columns. The column plate number for these columns varied from 2000-10 000, with peak skews of −0.2–1.7. Ten replicate sample injections were used on each column. to insure plausible statistics. A mobile phase flow-rate of 1.0 ml/min was used with the columns at ambient temperature.

RESULTS AND DISCUSSION

Computer simulation studies

Fig. 4 shows the precision and accuracy of calculating plate number for various τ/σ ratios (peak tailing) by the various methods with 2% random

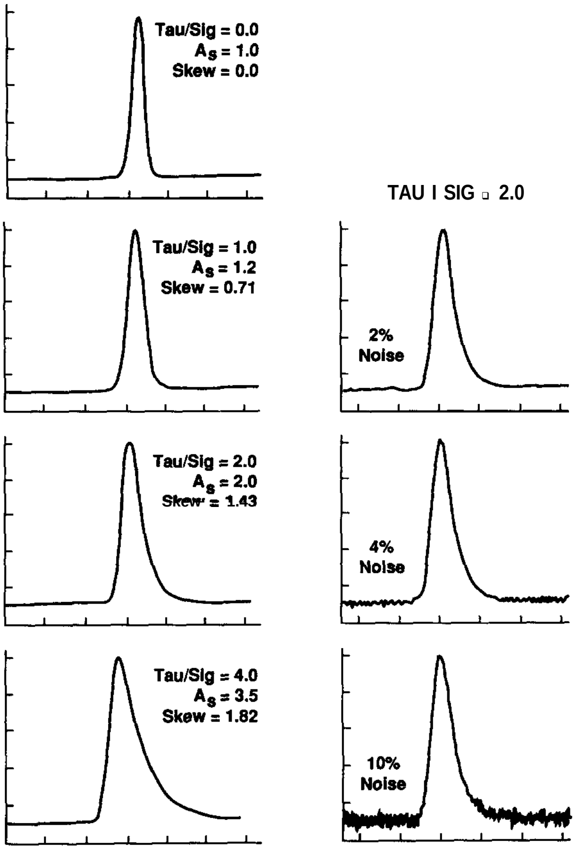

Fig. 2. Effect of peak tailing and noise.

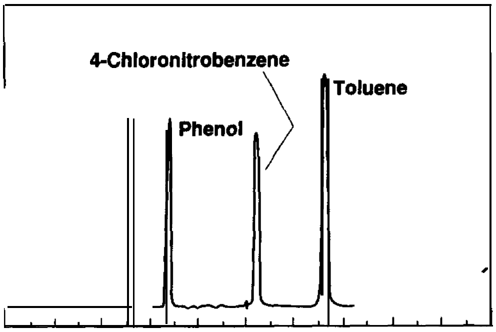

Fig. 3. Experimental test for plate number and peak shape. Columns and mobile phases: 2 Zorbax-C$_8$, 25 × 0.46 cm, methanol-water (80:20); 2 Zorbax-ODS, 25 x 0.46 cm, methanol–water (85:15); 1 Zorbax-PSM-60S, 25 x 0.62 cm, dichloromethane. Data points: 170 total, 10 replicate injections. Range: plates, 2000-20 000; skew, -0.2-1.7.

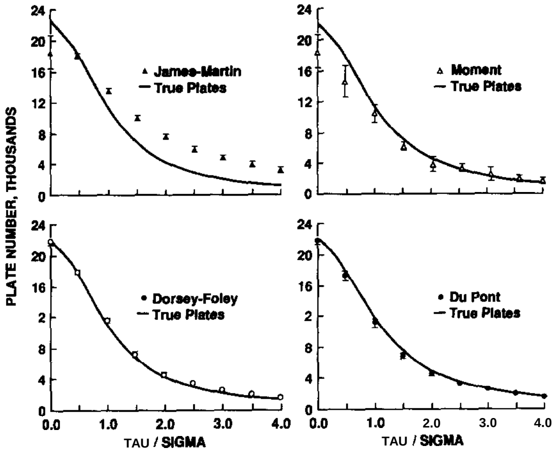

Fig. 4. Precision and accuracy of plate number measurements. 2% Random noise.

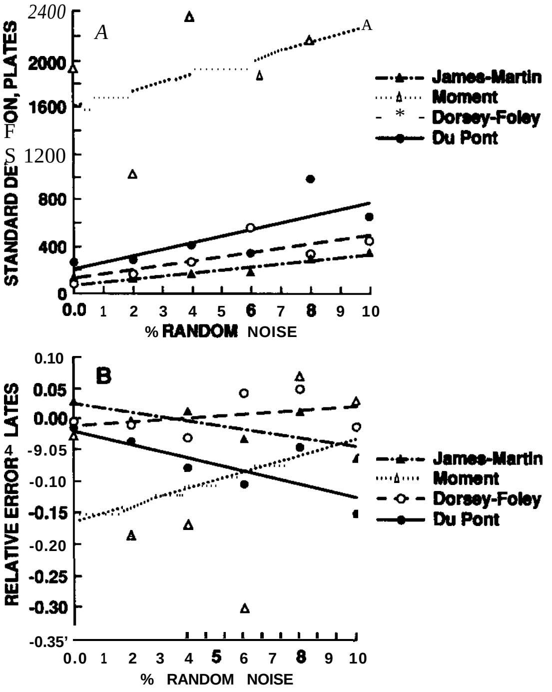

Fig. 5. Effect of noise on plate number measurements. $\tau/\sigma = 0.5$. (A) Precision measurements; (B) accuracy measurements.

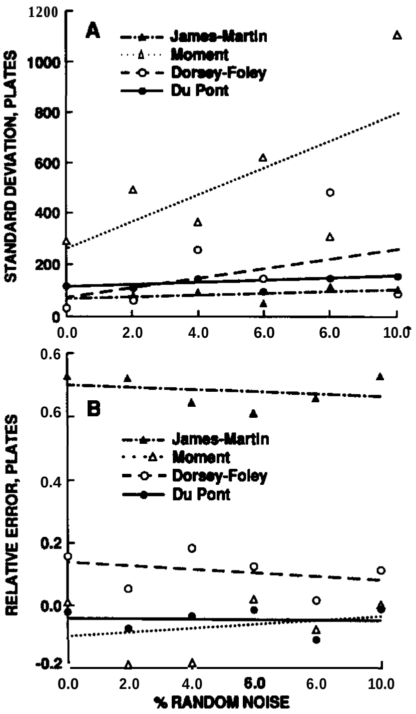

Fig. 6. Effect of noise on plate number measurements. $\tau/\sigma = 2.0$. (A) Precision measurements; (B) accuracy measurements.

noise imposed on the simulated chromatogram. These measured values are compared with the actual or true values used in the simulation. The J-M method provides precise measurements, but is increasingly inaccurate as increased peak tailing (increasing τ/σ ratio) occurs. This method shows ca. 15% error at a τ/σ ratio of 1.0, and a 60% error at a τ/σ ratio of 2.0. Similar results were obtained in a previous study [1]. The moment method shows poor precision and even poorer accuracy with small τ/σ values, because of a problem with the usual baseline bias presented by the computer algorithm. The percent error is about the same for increasing τ/σ values. For the same simulated peaks with 2% noise, the DF and DuPont methods both showed good precision and accuracy with increasing peak tailing.

Increasing the baseline random noise to 6% did not seriously change results from the J-M method from that seen at 2% noise -serious errors occur with increased peak tailing. However, the moment method shows much poorer precision with increased noise. The accuracy of measurements again appears similar for the DF and DuPont methods. But, precision of measurements of the DF method is degraded at higher baseline noise levels.

Fig. 5 compares the precision (Fig. 5A) and the accuracy (Fig. 5B) of plate number measurements made on tailing peaks with values of τ/σ = 0.5 with random baseline noise varying from 0–1%. Precision of measurements (Fig. 5A) are best for the J-M method, and about the same for the DF and DuPont methods throughout the range of noise studied. The precision of moment measurements is quite poor, particularly with more symmetrical peaks, presumably because of problems in establishing accurate baseline start and stop points for the peaks. With tailing peak shapes, the accuracy of plate number measurements (Fig. 5B) is best for the J-M method, with the DF and DuPont method slightly poorer. The moment method provides the most inaccurate results because of bias in the baseline-cutting algorithm.

Fig. 6 compares the accuracy and precision of plate number measurements made on tailing peaks

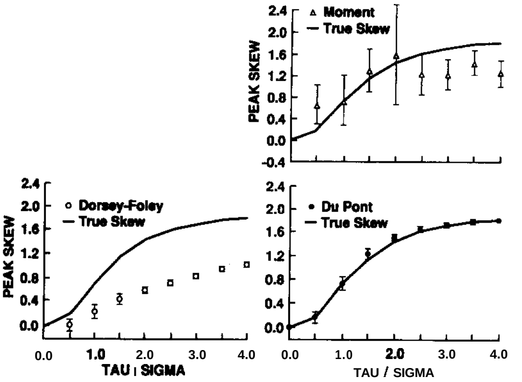

Fig. 7. Precision and accuracy of peak skew measurements. 2% Random noise.

with values of τ/σ = 2.0 and random baseline noise from 0–10%. These data show that the J-M method is the most precise (Fig. 6A) and by far the most inaccurate (Fig. 6B). The moment method is both inaccurate and imprecise with badly tailing peaks. The DF method and the DuPont methods have about the same precision, but the commercially-available DF method is less accurate with badly tailing peaks.

Fig. 7 illustrates the ability of methods to measure peak skew (directly related to τ/σ and peak symmetry values) precisely and accurately for simulated peaks with 2% random noise. (The J-M method is incapable of peak skew information because of the Gaussian-peak assumption.) The moment method is accurate but less precise at smaller τ/σ values; very poor precision and accuracy occur at large τ/σ ratios. The DF method is precise, but quite inaccurate with tailing peaks. The DuPont method shows both good accuracy and precision as peak tailing increases.

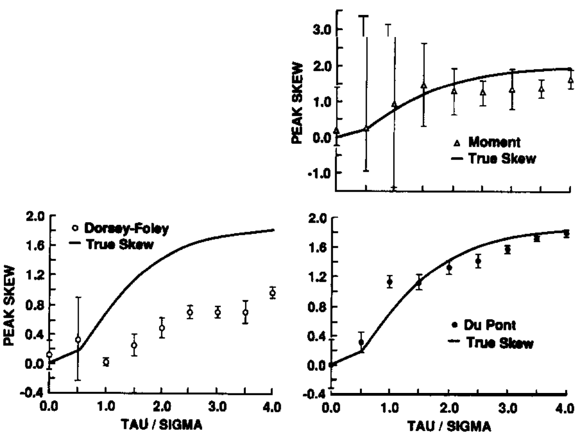

Fig. 8. Precision and accuracy of peak skew measurements. 6% Random noise.

Increasing the random noise to 6% causes no significant change in the accuracy of the moment method, as shown in Fig. 8. However, method precision is further degraded. The precision of the DF method is about the same as with lower baseline noise, but accuracy is poorer. Increased noise does not perceptibly change the precision of the DuPont measurements, and accuracy is only slightly degraded.

It is important to note that Figs. 7 and 8 suggest that the DF method is inaccurate for peak skew measurements (errors exceeding 60% for $\tau/\sigma > 2$). These results contradict those from other studies regarding the level of accuracy with the DF method [6]. Since other investigators have not reported the DF equation to be inaccurate for peak skew [8], we suspect that the source of inconsistency is the commercial software available for this study.

Chromatographic peak study

Table II summarizes the experimental data obtained with the four peak characterization methods on seventeen different columns having widely varying plate numbers and peak tailing or peak skew values. The ± values in this table represent the data spread (standard deviation) obtained with ten replicate sample separations.

Fig. 9 shows the plate number calculations from these column tests arbitrarily plotted against measured moment values. (True values are unknown and the moment method probably is the most accurate.) The intercept of these plots illustrate the accuracy of the measurements, while the spread of the values provides information on precision. The J-M method shows accuracy problems, since the plot does not intercept the origin. The DF and the DuPont methods show about the same accuracy and precision for plate number throughout the range of column studied. These results check well with results obtained by computer simulation.

Data in Fig. 10 compare peak skew calculations from the column tests. These results show that the

TABLE II

TEST RESULTS: SUMMARY

$\sigma_{av.}$ = Average of individual σ values, an index that reflects the susceptibility of each method to baseline noise; r = correlation **coefficient** between an individual method referenced against the moment calculation; $\sigma_{yy'}$ = standard deviation from the best-fit linear-regression line between each method versus the moment calculation; a, b = intercept and slope of the best-fit line, y = a + bx.

LC	Plates ($N_{av.}$ ± a)				Skew (Sk.,, ± σ)			
	J-M	DF	DuPont	Moment	DF-NA	DF	DuPont	Moment
Column-peak								
A-1	6470 ± 260	5360 ± 230	6290 ± 220	5440 ± 190	0.54 ± 0.22	0.34 ± 0.14	0.14 ± 0.06	**−0.01 ± 0.04**
2	7310 ± 320	**6600 ± 210**	7330 ± 174	**6340 ± 210**	0.43 ± 0.18	**0.17 ± 0.10**	0.06 ± 0.04	-0.01 ± 0.04
3	7830 ± 320	7690 ± 140	7690 ± 400	6263 ± 110	**0.10 ± 0.06**	0.01 ± 0.04	-0.07 ± 0.10	**0.04 ± 0.11**
4	9180 ± 140	8832 ± 170	8720 ± 290	7460 ± 710	0.02 ± 0.05	-0.05 ± 0.04	-0.21 ± 0.10	**−0.17 ± 0.06**
B-1	10 750 ± 160	7490 ± 510	8490 ± 240	8650 ± 190	1.07 ± 0.47	0.77 ± 0.28	0.80 ± 0.05	0.76 ± 0.03
2	15 110 ± 130	11 360 ± 550	12 180 ± 400	12 570 ± 810	1.34 ± 0.35	0.72 ± 0.19	0.74 ± 0.08	0.72 ± 0.23
3	19 110 ± 1010	16 990 ± 1400	16 920 ± 310	16 160 ± 340	0.58 ± 0.22	0.38 ± 0.10	0.52 ± 0.04	0.68 ± 0.07
4	22 280 ± 170	19 408 ± 610	20 930 ± 240	19 450 ± 430	0.56 ± 0.21	0.23 ± 0.05	0.27 ± 0.03	0.51 ± 0.07
C-1	10 810 ± 190	6890 ± 230	7020 ± 250	6880 ± 340	**1.49 ± 0.17**	**1.12 ± 0.04**	1.22 ± 0.04	1.24 ± 0.04
2	14 210 ± 290	9750 ± 210	10 220 ± 300	10 070 ± 380	1.34 ± 0.22	0.92 ± 0.08	1.02 ± 0.04	1.06 ± 0.04
3	20 050 ± 230	15 330 ± 140	16 480 ± 420	15 600 ± 730	0.95 ± 0.23	**0.50 ± 0.15**	0.69 ± 0.05	0.79 ± 0.05
4	23 330 ± 170	19 180 ± 170	20 210 ± 540	19 280 ± 188	0.61 ± 0.15	0.50 ± 0.10	0.53 ± 0.06	0.67 ± 0.10
D-1	3660 ± 130	1870 ± 80	2040 ± 80	1950 ± 70	1.57 ± 0.07	1.53 ± 0.05	1.48 ± 0.05	1.49 ± 0.06
2	5302 ± 160	2460 ± 120	2820 ± 140	2700 ± 140	1.63 ± 0.05	1.58 ± 0.03	1.53 ± 0.02	1.44 ± 0.03
3	8520 ± 290	**4200 ± 210**	4700 ± 410	4130 ± 720	1.57 ± 0.09	1.47 ± 0.06	1.48 ± 0.08	**1.49 ± 0.37**
4[a]	9940 ± 300	5220 ± 280	4900 ± 380	**3130 ± 380**	1.52 ± 0.05	1.41 ± 0.06	1.61 ± 0.05	2.24 ± 0.10
E-1”	19 910 ± 280	8320 ± 230	8440 ± 360	5070 ± 270	1.57 ± 0.08	1.54 ± 0.05	1.74 ± 0.02	1.94 ± 0.01
Statistics								
$\sigma_{av.}$	270	400	300	370	0.170	0.090	0.053	0.085
r	0.985	0.992	0.996	1	0.932	0.944	0.984	1
$\sigma_{yy'}$	1070	710	540	0	0.194	0.173	0.100	0
a	1888	163	312	0	0.272	0.032	-0.039	0
b	1.088	0.986	1.031		0.910	0.908	1.008	1

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[a] Unusual peak shape, not included in statistics; data plotted as cross (×) in summary plots.

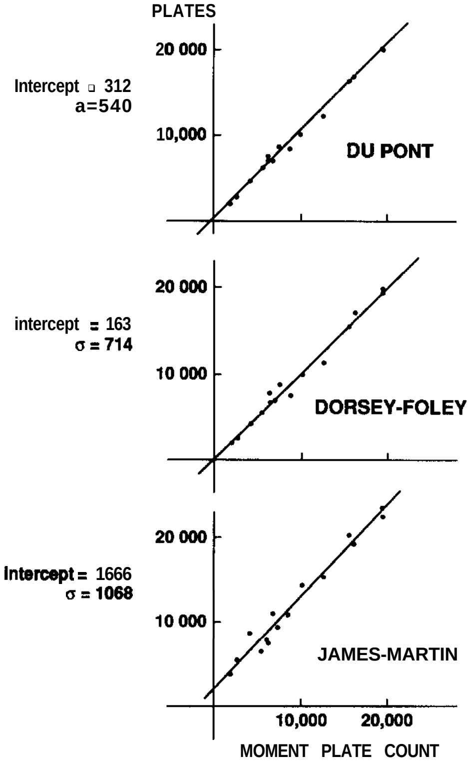

Fig. 9. Plate number calculations from column test experiments,

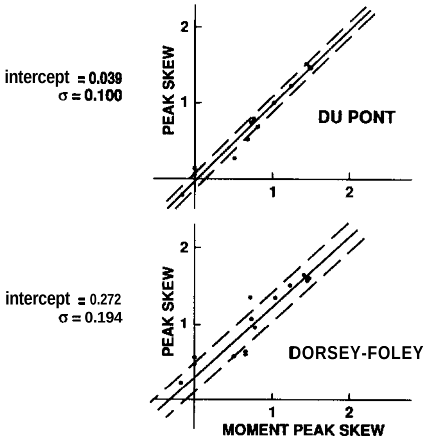

Fig. 10. Peak skew calculations from column test experiments.

DF method has a positive bias; the plot intercepts the ordinate above the origin. The accuracy and precision of the DF method is about two-fold poorer than the DuPont method, as suggested by data in the Fig. 10B plots.

Table III ranks the peak characterizing methods based on the experimental separations. These results are based on replicate columns of five different types, using ten replicate runs with four solutes. Ranking numbers show that for plate number calculations, precision is best for J-M, closely followed by the DuPont method. The DF and the moment methods are 25–30% less precise. Both the DF and DuPont methods show good correlation with the moment values. Accuracy rankings show DF best, closely followed by DuPont; J–M is by far the least accurate for these “real” chromatographic peaks.

TABLE III

PEAK ANALYSIS RESULTS ON EXPERIMENTAL SEPARATIONS

Data from 5 columns, 4 solutes, 10 replicate runs each.

Computer analysis method	Plate number (rank”)		Peak skew (rank”)		Final ranking
	Precision, σ	Accuracy, AN	Precision, σ	Accuracy, ΔS	
James-Martin	270 (1)	1888 (4)	– (4)	– (4)	4
Moment	370 (3)	[b] (3)	0.09 (2)	[b] (3)	3
Dorsey–Foley	400 (4)	161 (I)	0.17 (3)	0.027 (2)	2
DuPont	300 (2)	312 (2)	0.05 (I)	-0.04 (1)	I

[a] 1 = Most favorable; 4 = least favorable.

[b] Moment analysis used as reference value on completely separated peaks; moment analysis may not be accurate as a reference at low signal-to-noise (S/N) ratios,

TABLE IV

CONCLUSIONS ON COMPUTER MEASUREMENT METHODS FOR EXPERIMENTAL PEAKS

Criteria		James-Martin	Moment	Dorsey-Foley	DuPont
Plates’	Precision	+ + +	0	+ + +	+ +
	Accuracy	− −	+	+ + +	+ + +
Skew”	Precision	− − −	- -	+ + +	+ + +
	Accuracy	− − −	+ +	- - -	+ + +
Overall rating			0	+ +	+ + +

[a] $\tau/\sigma \leqslant 1$.

For measuring peak skew, the $\sigma_{av.}$ data in Table 11 show that the DuPont method is about 70% more precise than either the DF or moment method; J-M ranks last because of its inability to measure peak skew. The data in Table II further show that the DuPont method is more accurate in peak skew measurements. The correlation plots show $\sigma_{yy'}$ values about half that of the DF method measured by the commercial software. The slope of the linear regression line for the DuPont method shows only a 1% error from the ideal value of 1 .O. The slope of the data for the DF method shows a 9% error from ideal. Poorest results are for the DF-NA method; the linear regression plot does not pass through the origin, resulting in skew accuracy bias.

CONCLUSIONS

A final ranking of the various computer methods for measuring plate number and peak skew or peak tailing depends on the level of background noise and the amount of peak tailing. Table IV summarizes our conclusions regarding the four methods studied, arbitrarily assuming peaks with $\tau/\sigma \leqslant 1$ (peak skew ~0.71). The moment method is generally impractical in most real separations because of potential peak overlap problems, and difficulties in establishing accurate baseline “cut-points”. The J-M method is most precise for measuring plate number, but should be used only with highly symmetrical peaks. The J-M method is incapable of measuring peak skew. Both the DF and the DuPont methods are competent for measuring the plate number and skew of peaks with good symmetry and low background noise. With increased baseline noise or more peak tailing, the DuPont method appears most satisfactory. The DuPont method has been used successfully in our laboratories for about four years in a variety of separations, including HPLC, GC, size-exclusion chromatography and field flow fractionation.

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