Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Comparisons of Methods for Calculating Retention and Separation of Chromatographic Peaks

R. E. Pauls[a b]; L. B. Rogers[a]
[a] DEPARTMENT OF CHEMISTRY, UNIVERSITY OF GEORGIA, ATHENS, GEORGIA [b] Amoco Research Center, Naperville, Illinois

To cite this Article Pauls, R. E. and Rogers, L. B.(1977) 'Comparisons of Methods for Calculating Retention and Separation of Chromatographic Peaks', Separation Science and Technology, 12: 4, 395 — 413
To link to this Article: DOI: 10.1080/00372367708058085
URL: http://dx.doi.org/10.1080/00372367708058085

SEPARATION SCIENCE, 12(4), pp. 395–413, 1977

Comparisons of Methods for Calculating Retention and Separation of Chromatographic Peaks

---

R. E. PAULS* and L. B. ROGERS

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF GEORGIA
ATHENS, GEORGIA 30602

Abstract

The accuracy and precision of calculating retention times from means and peak maxima have been examined using an exponentially modified Gaussian as a model for tailed chromatographic peaks. At different levels of random noise, retention times could be determined with nearly the same precision using either the mean or maximum. However, the accuracies and precisions of the maxima were affected by the number of points used in the digital smooth and by the number of points recorded per unit of standard deviation. For two peaks of similar shape, consistency in the selection of points should usually permit differences in retention to be determined accurately and with approximately the same precision using maxima, means, or half-heights on the leading side of the peak.

INTRODUCTION

The retention time or volume is a parameter of great interest in chromatographic experiments since it is characteristic of the eluting species. The retention time of the chromatographic peak is normally determined by calculating either the peak mean, which is equivalent to the first statistical moment, or the peak maximum. Statistical moments have been widely used to characterize chromatographic peak shapes (*1*) because they

*Current address: Amoco Research Center, P.O. Box 400, Naperville, Illinois 60540.

completely specify the peak. The zeroth moment is equal to the peak area. The first moment is the center of gravity, or peak mean, and is equivalent to the retention time of the peak. The first moment is of fundamental significance for gas–solid chromatography since it depends only on the distribution ratio, the carrier-gas velocity; it is independent of rates of mass transfer and radial diffusion (*2*). For that reason, only the value of the peak mean should be used in calculations of partition coefficients. The second moment about the peak mean is a measure of the peak width. The third and fourth moments are related to the peak skew and excess, respectively.

Shifts in baseline, the need for a finite cutoff of data following a peak (*3*), and random noise can all affect the accuracy and precision of moment calculations. Chesler and Cram (*4*) have studied the effects of peak sensing and noise on the precision and accuracy of moments. They have shown the importance in moment calculations of using wide integration limits and a large number of data points across a peak.

An alternate method of calculating retention time is to measure the peak maximum. The peak maximum is usually easier to measure although it lacks the theoretical significance of the first moment and can be influenced by kinetic, as well as thermodynamic, effects. The peak maximum can be obtained by least-squares curve fitting about the top of the peak. The simpliest approach to curve fitting is to use a second-order least-squares fit. Once the coefficients of the second-order equation have been determined, the expression can be differentiated to obtain the maximum.

Chromatographic peak shapes are often assumed to be described by a simple Gaussian distribution. In practice, peaks deviate from that simple model. Dead volume and other extra-column effects have been shown to alter a peak exponentially (*5–7*). Instrumental factors such as electronic time constants and detector systems will also exponentially convolute a Gaussian peak (*8*). Therefore, a more appropriate model with both theoretical and experimental justification is an exponentially modified Gaussian which can be generated by convoluting a Gaussian with an exponential decay. Gladney et al. (*9*) and Littlewood and co-workers (*10*) have used an exponentially modified Gaussian in deconvolution studies. McWilliam and Bolton (*8*) also used such a model to study the effects of instrumental distortion on peak shape and separation. Most recently, Grushka used an exponentially convoluted Gaussian in a study on the characterization of strongly overlapped peaks (*11*).

An exponentially modified Gaussian can be generated by the following expression:

$$f(t) = \frac{A}{\tau\sigma\sqrt{2\pi}} \int_0^\infty \exp\left[-\frac{(t - t_R - t')^2}{2\sigma^2}\right] \exp\left[-\frac{t'}{\tau}\right] dt' \qquad (1)$$

where A is the peak amplitude, σ is the standard deviation of the Gaussian, τ is the time constant of the exponential decay, t_R is the first moment of the Gaussian, and t' is a dummy variable of integration. The area of this expression is always that of the original Gaussian, and the maximum of the convoluted expression will always fall on the original Gaussian curve. The first moment of the convoluted Gaussian is given by

$$M_1 = t_R + \tau \qquad (2)$$

The second through fourth moments of the modified Gaussian are functions of σ and τ and were derived by Grushka (*11*):

$$M_2 = \sigma^2 + \tau^2 \qquad (3)$$

$$M_3 = 2\tau^3 \qquad (4)$$

$$M_4 = 3\sigma^4 + 6\sigma^2\tau^2 + 9\tau^4 \qquad (5)$$

The purpose of the present study was to compare the precision and accuracy of retention times calculated as the peak means and as the peak maxima using simulated data. We have also examined the precision and accuracy of calculating the small differences in retention, or peak separation, for separate chromatograms. Retention differences were calculated from peak means, from peak maxima, and from differences in retention at the peak half-heights (*12*). The last method was used by Shepard et al. (*12*) who reported that small peak separations of isotopically substituted molecules could be measured more precisely in that way. In our study, a comparison has also been made between calculating peak variance as the second moment and from the width at half height for peaks of varying amounts of skew.

EXPERIMENTAL

All the simulations used in this study were carried out on a PDP 11/20 minicomputer (Digital Equipment Corp., Maynard, Massachusetts) and programmed in BASIC language. Simulated chromatographic peaks were generated using the following modification of Eq. (1) (*13*):

$$f(t) = \frac{A}{\tau\sigma\sqrt{2\pi}} \int_0^\infty \exp\left[-\frac{(t - t_R{}^0 + \tau - t')^2}{2\sigma^2}\right] \exp\left[-\frac{t'}{\tau}\right] dt' \qquad (6)$$

where $t_R{}^0$ is the first moment of the convoluted Gaussian peak. All other variables are the same as in Eq. (1). Throughout the remainder of this paper, we shall use σ as it is represented in Eqs. (1) and (6).

Either 240 or 480 points were generated for each peak, depending upon the width of the peak and the amount of peak tailing. Chromatograms were usually generated with the same first moment which was located at the 120th data point. However, for the studies on differences in retention times, a pair of peaks was generated which had their first moments one data point apart. The simulated chromatograms had σ values ranging from 5 to 40 data points and τ/σ values ranged from 0.1 to 2.0. Peaks with larger values of τ/σ are more tailed, so larger ratios reflect greater peak asymmetry.

Random noise was generated using the random-number generator in DEC BASIC in conjunction with the "RANDOMIZE" function, which insures that the array of random numbers in BASIC is accessed at a random entry point. Peak-to-peak noise values of $\pm 0.15\%$, $\pm 0.50\%$, $\pm 1.5\%$, and $\pm 5\%$ of the peak amplitude were used to represent extremes from low noise to very high noise levels. In all simulations with noise, 10 runs were made from which the mean and the standard deviation were calculated.

The peak maximum was calculated by least-squares fitting (*14*) the top of the peak with the quadratic function

$$f(t) = a_2 t^2 + a_1 t + a_0 \tag{7}$$

In order to least-squares fit the peak, it was first necessary to locate the highest data point, then a least-squares fit was centered about that point. In this study, fits ranging over the odd numbers from 3 to 55 data points were performed. The fit was carried out using the technique of Grams polynomials (*15*) in which the coefficients a_2, a_1, and a_0 were easily calculated. Once the coefficients in Eq. (7) had been determined, the expression was differentiated, the resulting expression equated to zero, and the location of the peak maximum and the peak height were calculated. The true value of the peak maximum was determined by differentiating Eq. (6) and numerically solving for the maximum.

The *n*th moment is defined by

$$M_n = \frac{\int_0^\infty t^n f(t)\, dt}{\int_0^\infty f(t)\, dt} \tag{8}$$

Moments higher than the first were calculated about the first moment by

$$M_n = \frac{\int_0^\infty (t - M_1)^n f(t)\, dt}{\int_0^\infty f(t)\, dt} \tag{9}$$

A summation replaced the integral sign in moment calculations in our study. The beginning and cutoff limits for the summation of the moments were determined using a thresholding technique similar to that used by Chesler and Cram (*4*). A threshold equal to the noise level was used to initiate and terminate the summation. Baseline points before and after the threshold were not included.

In calculations to determine the retention time at the half height, it was first necessary to obtain the peak height by least-squares fitting the top of the peak. Then the half height was located and a 6-point first-order least-squares fit was carried out about the 3 points on each side of the half height. The retention time at half height was then determined using the slope and intercept obtained in the least-squares fit and the value of the half height.

The width at half height was approximated by locating the data points on either side of the half height on both sides of the peak and calculating the time at the half height from the slope between the two points. The width was then obtained by subtraction.

RESULTS

The effect of increasing the τ/σ ratio on the shape of a chromatographic peak is shown in Fig. 1. It should be noted that the maxima of the convoluted peaks were on the original Gaussian curve and that the areas under the curves were normalized.

Peak Maximum

In order to find the optimal conditions for the least-squares fitting about the top of a peak, the effects of random noise, peak tailing, and peak width on the accuracy and precision of calculating the time of the peak maximum were determined. The error in calculating the time of the peak maximum was evaluated by determining the difference between the time calculated from the least-squares fit and the true peak maximum as determined by the first derivative of Eq. (1). The precision in the presence of noise was evaluated by determining the standard deviation of 10 runs as previously discussed.

The effects of the level of random noise on the accuracy of the curve fitting are shown in Fig. 2, which is a plot of the error in the maximum vs number of data points used in the smooth. The peak had a σ value of 20 points and a τ value of 10 points, corresponding to a τ/σ value of 0.5. With no added noise, the error increased in a positive direction with the number of points fitted, indicating that the calculated retention time was

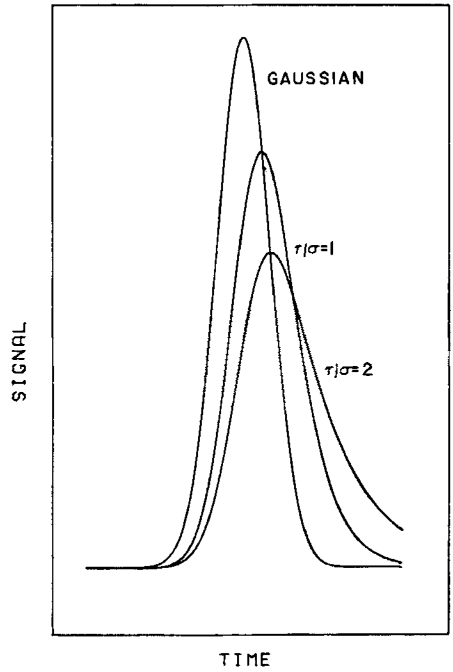

FIG. 1. Effect of τ/σ on peak shape.

increasing as more points were fit. The difference between a 7-point and a 55-point fit was 0.3 data points corresponding to an error of 0.015 σ units. As random noise was added to the peak, the error became larger when smaller numbers of points were fit, but it tended to fall in line with the "no-noise" case when more points were fitted.

The effect of random noise on the precision of the fit is shown in Fig. 3, where the standard deviation of 10 runs with added noise is plotted against the number of points fit. This peak also had a σ of 20 points and a τ of 10. The first thing to note about this data is the existence of a minimum in all four curves. When only a few points were fit, the precision was relatively poor; it then decreased rapidly, reached a minimum and gradually increased. The number of points that produced the minimum depended upon the random noise level. For a noise level of ±0.15%, the minimum of 19 points fitted corresponded to a fit of ±0.50 σ about the maximum, while for a noise level of ±0.50%, the minimum shifted to a 29-point fit or ±0.75 σ about the maximum. As expected, the value of

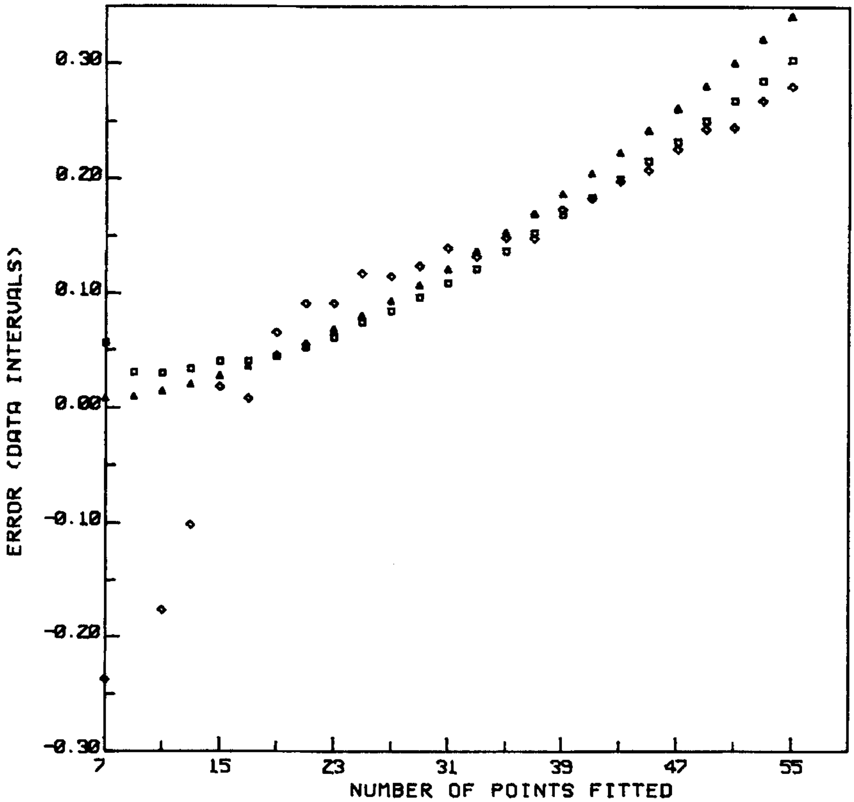

FIG. 2. Effect of random noise on the accuracy of the peak maximum as a function of the number of points fitted for τ/σ of 0.5: (△) 0% noise, (□) ±0.15% noise, and (◇) ±1.5% noise.

the standard deviation or the uncertainty in the maximum increased as the level of random noise increased.

The effect of peak width (or more properly, the number of points per unit of standard deviation) on the calculation of the peak maximum was evaluated by varying the value of σ for a peak while keeping the τ/σ ratio constant. Using τ/σ of 0.5, the error in data point intervals for the calculated peak maximum with σ values ranging from 5 to 40 is shown in Fig. 4. Again, the error increased in a positive direction as the number of fitted points was increased. Initially, the rate of increase was inversely proportional to the value of σ. For a peak with a σ of 20 points, the dif-

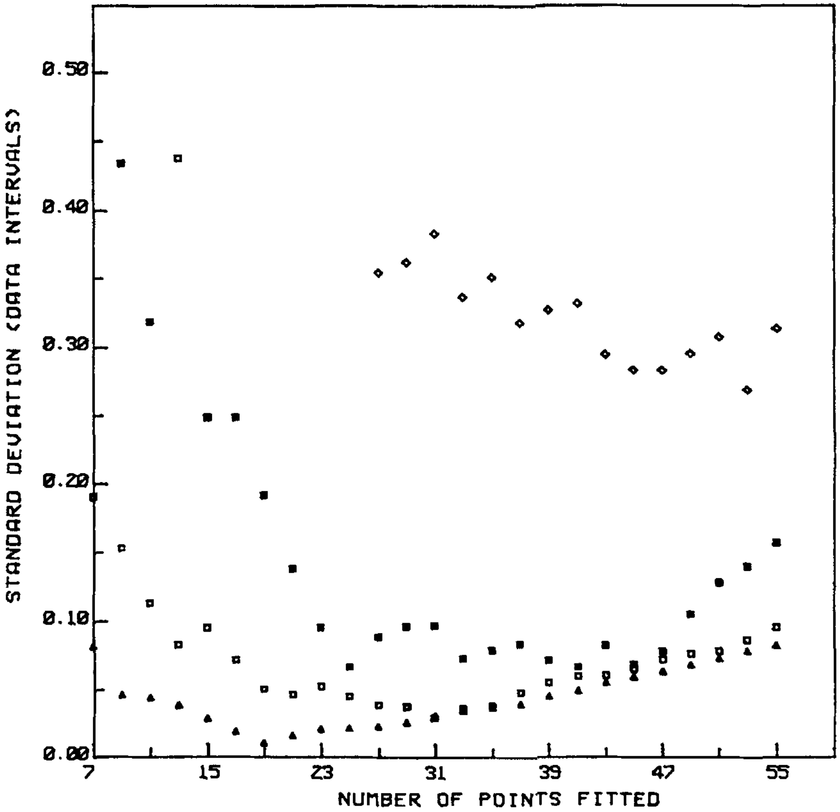

FIG. 3. Effect of random noise on the precision of the peak maximum as a function of the number of points fitted for τ/σ of 0.5: (△) ±0.15%, (□) ±0.50%, (■) ±1.50%, and (◇) ±5.00%.

ference between a 7- and a 31-point fit was 0.12 data intervals or 0.006 σ units, while for a peak with a σ of 5, the difference was 0.26 data intervals or a change of 0.05 σ units. For peaks having a small value of σ, the error function leveled off at higher numbers of points fitted because the baseline was being included in the fit.

The precision of the fit was also affected by the number of points used in the fit as shown in Fig. 5. At a noise level of ±0.15%, the precision once again passed through a minimum which now depended upon the σ value of the peak. For a peak having a σ value of 10 points, the minimum was located at a 15-point fit which corresponded to a fit over ±0.75 σ units

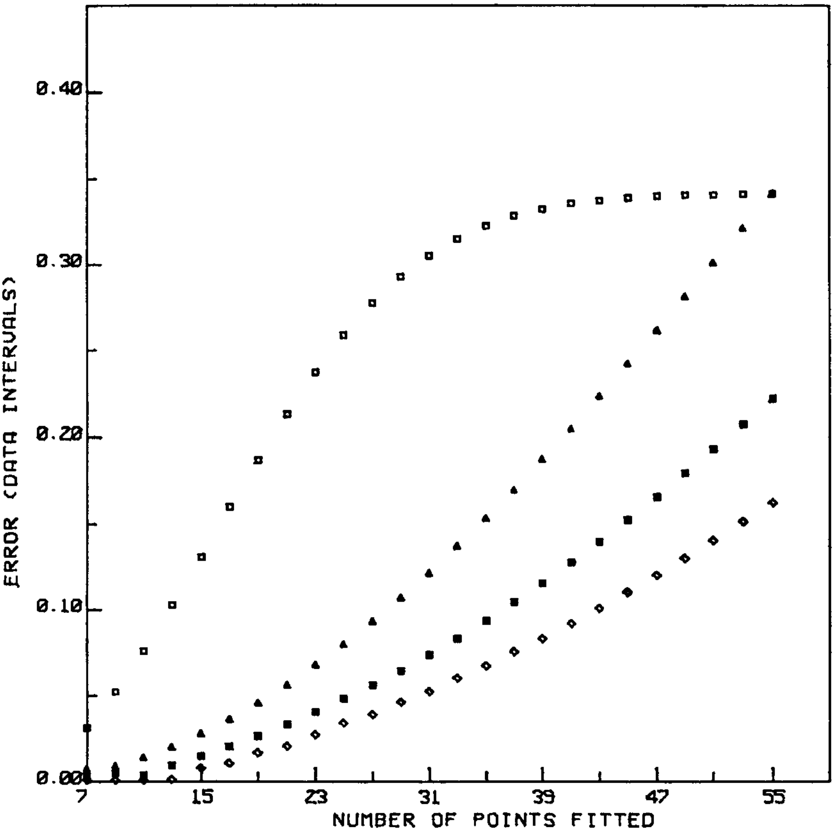

FIG. 4. Effect of number of points per standard deviation unit on the accuracy of the peak maximum as a function of the number of points fitted for τ/σ of 0.5: (□) $\sigma = 5$, (△) $\sigma = 10$, (■) $\sigma = 30$, and (◇) $\sigma = 40$.

about the maximum. For a peak with a σ of 20 points, the minimum was located at 19 points which corresponded to a fit over ±0.50 σ units. The minima for peaks with σ values of 30 and 40 points were also located at fits over ±0.50 σ units. As the value of σ increased, the valley of the minimum became broader. The standard deviation of the peak maximum time at the minimum was about ±0.02 data points, independent of the value of σ, for a noise level of ±0.15%. Therefore, the uncertainty in σ units was smallest for the largest value of σ.

Peak tailing, as reflected by the τ/σ ratio, had a large effect on the ac-

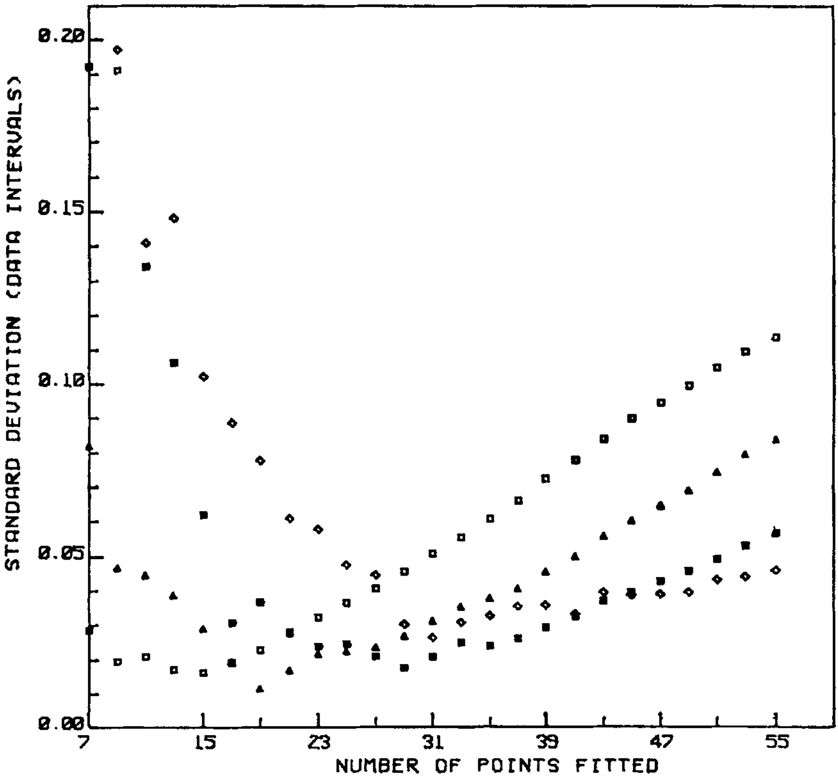

FIG. 5. Effect of number of points per standard deviation unit on the precision of the peak maximum as a function of the number of points fitted for τ/σ of 0.5 and a noise level of 0.15%: (□) $\sigma = 10$, (△) $\sigma = 20$, (■) $\sigma = 30$, and (◇) $\sigma = 40$.

curacy of the calculated peak maximum. Figure 6 is a plot of the error in calculated maximum vs the number of points fit where peaks had a σ of 20 points and no added noise. The error in the calculated maximum increased rapidly as the peak became more tailed. For a peak with a τ/σ ratio of 2, the difference between a 7- and a 39-point fit was 0.9 data units, corresponding to 0.045 σ units. As the τ/σ ratio approached zero, the error also became smaller until, in the limit for a Gaussian curve, the error was zero and was independent of the number of points used in the fit.

The value of the τ/σ ratio had little effect on the precision of the fit as

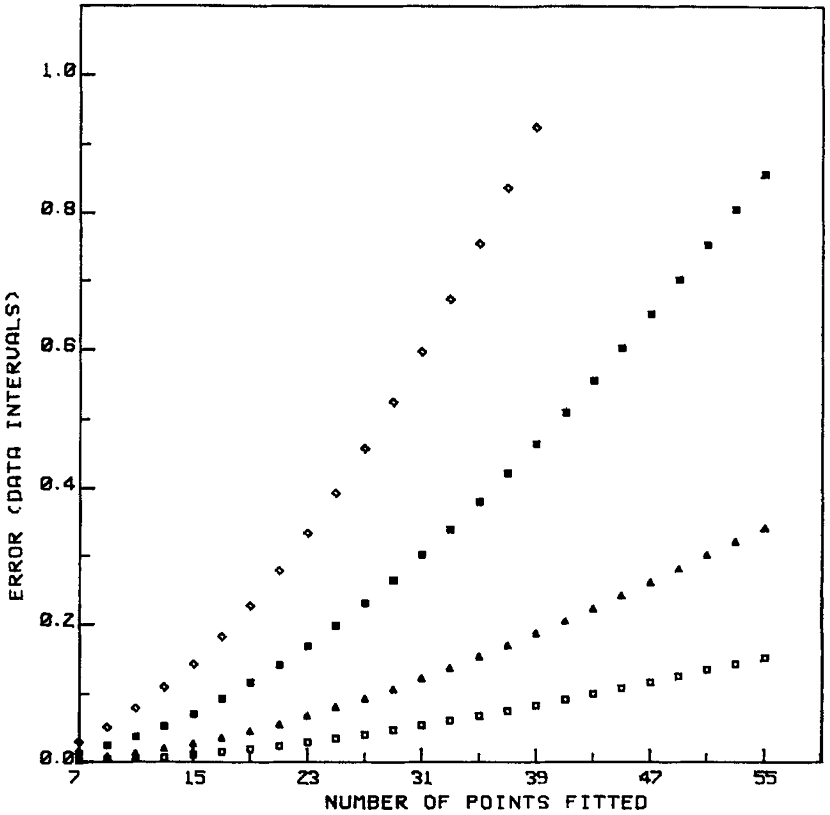

FIG. 6. Effect of peak tailing on the accuracy of the peak maximum as a function of the number of points fitted for a σ of 20: (□) $\tau = 6$, (△) $\tau = 10$, (■) $\tau = 20$, and (◇) $\tau = 40$.

can be seen in Fig. 7. The location of the minimum did not vary significantly as τ was changed from 10 to 40.

The accuracy in the calculation of the peak maximum was affected by many factors. As more points were used to fit the skewed peak, the error increased in a positive direction to produce a longer retention time. The rate of increase was particularly dependent upon the τ/σ ratio; the larger the value of τ/σ, the greater the increase. For a constant τ/σ ratio, the rate of increase was larger for peaks having a smaller value of σ. When random noise was added to the peak, better accuracy was obtained by fitting over more points.

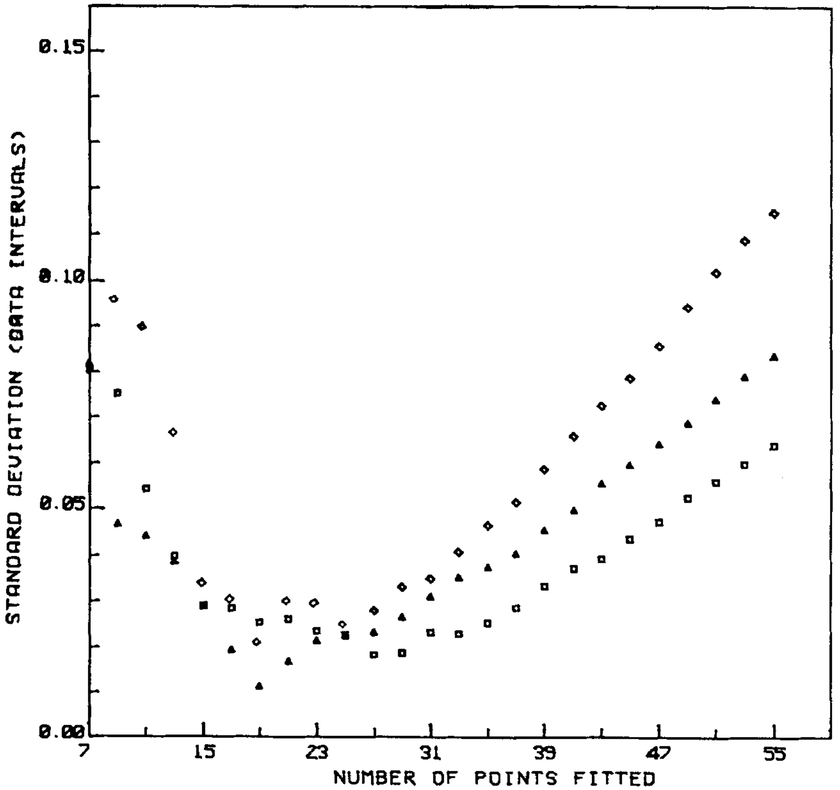

FIG. 7. Effect of peak tailing on the precision of the peak maximum as a function of the number of points fitted for a σ of 20 and a noise level of 0.15%: (△) $\tau = 10$, (□) $\tau = 20$, and (◇) $\tau = 40$.

The precision of the fit went through a minimum. The location of the minimum was determined by two factors. The first was the level of the random noise and the second was the value of σ. Both an increase in the noise level and an increase in the value of σ shifted the minimum to a larger number of fitted points. However, the value of τ/σ had little effect on the location of the minimum in terms of σ units.

Therefore, in order to obtain the best possible accuracy in calculating the peak maximum, it is necessary to fit as small a number of points as possible. If too few points are used, however, the precision will suffer. For peaks with a good signal-to-noise ratio, on the order of 100, a fit over ±0.5 σ units will give good precision while at the same time giving reasonably good accuracy. For badly tailed peaks, better accuracy may be obtained by using a smaller number of points while, for cases with lower signal-to-noise ratios, it would be advisable to use a larger number of points so as to obtain reasonable precision. In all subsequent calculations of peak maximum, the fit that gave the best precision was used.

Comparison of Methods for Calculating Retention

The effects of the level of random noise on the calculation of retention time and differences in retention time are summarized in Table 1 for a peak with a σ of 20 points and a τ value of 10 points. Retention times were calculated using both the peak maximum and the peak mean. Differences in retention times were calculated using those two measures of retention and the retention times at the half height.

As discussed earlier, there was a difference between the retention times calculated as the peak maximum and the peak mean. For our skewed peaks, the time for the peak maximum was always less than the time for the peak mean. For the peaks in Table 1, with no added noise, the difference between the maximum and the mean was about 1.4 data intervals. The value of the peak maximum increased with noise because a larger number of points was used to fit the peak as discussed earlier. The value of the peak mean decreased with increasing noise because the higher noise levels required a higher threshold for initiating and terminating the moments. As a result, more of the tail was lost than the leading side, so that the first moment shifted to lower values. Therefore, although the values approached one another, the errors in calculating the retention times for both the peak mean and the peak maximum increased as the noise level increased. However, all three methods for calculating the difference in retention gave values which were not statistically different at the 95% confidence level.

As expected, the precision in calculating both the retention time and the differences in retention became worse as the noise level increased. Based on the F test with a 95% confidence limit, there was no statistical difference in the precision of the retention or in the differences in retention. For a noise level of ±0.15%, the retention was reproducible to about ±0.020 data units or ±0.001 σ units, while differences in retention were reproducible to ±0.030 data points or 0.0024 σ units. Thus for a noise level of ±0.15%, it was possible to determine a 1-unit difference in retention with a precision of 2%. As the noise increased to ±1.5%, the values

TABLE 1

Effect of Random Noise on the Accuracy and Precision of Calculating Retention and Differences in Retention[a]

Noise (%)	Peak maximum	Peak mean	Difference in maximum	Difference in mean	Difference at half height
0	117.571	119.000	1.000	1.000	1.000
±0.15	117.630 ± 0.024	118.977 ± 0.013	1.001 ± 0.040	0.996 ± 0.021	1.009 ± 0.025
±0.50	117.693 ± 0.026	118.904 ± 0.033	0.978 ± 0.056	0.997 ± 0.033	1.019 ± 0.067
±1.50	117.596 ± 0.091	118.762 ± 0.098	1.016 ± 0.165	0.922 ± 0.130	1.015 ± 0.091
±5.0	117.798 ± 0.223	118.141 ± 0.294	0.983 ± 0.236	1.113 ± 0.367	1.264 ± 0.788

[a] $\sigma = 20$ points, $\tau = 10$ points.

for retention and for retention difference increased to ±0.0045 and ±0.0065 σ units, respectively. Hence the uncertainty in a 1-unit retention difference increased to 13% for ±1.5% noise; it increased to 37% for ±5% noise.

In Table 2 are given the values for the retention times and differences in retention times as the value of σ was varied. The τ/σ ratio was kept constant at 0.5. While the peak mean remained constant as σ increased, the peak maximum decreased to lower absolute values. However, in terms of σ units, the peak maximum and peak mean were always a fixed distance apart. There was no significant difference in the precision of the two methods for calculating retention and for the three methods for calculating differences in retention. In terms of data interval units, the precision in calculating the retention became worse as the value of σ increased; however, in terms of σ units, the precision improved from ±0.004 σ units for a peak with a σ value of 5 to ±0.0016 σ units for a peak with a σ value of 40. The same trend was observed for differences in retention.

The influence of peak tailing on the retention calculation is summarized in Table 3 where all peaks had a σ value of 20 points and a noise level of 0.15%. As the peaks become more tailed, the peak maximum shifted to shorter times (for a constant mean) so the peak mean appeared fairly constant.

As the value of τ increased, the difference between the peak maximum and the peak mean became very large. For a τ of 40 points and τ/σ of 2, the difference was close to 20 points or 1 σ unit. The accuracy in calculating the first moment fell off for higher values of τ. Again, this was because of the thresholding technique used to calculate the moments. For badly tailed peaks, the contribution to the first moment from the tail was increased and early termination of the summation shifted the first moment to lower values.

There was again no significant difference in precision for the calculation of the retention time or the differences in retention times. The precision values for the retention time tended to increase slightly in terms of data-interval units as the value of τ increased, from about ±0.015 data points for a peak with a τ of 2 to ±0.040 units for a peak of a τ of 40 points. In terms of the standard deviation units (second moment), the difference remained fairly constant at about ±0.001 units. The precision in the calculation of the differences in retention followed the same general trend. It was again possible to calculate a 1-unit difference in retention with a precision of 1 to 2%.

TABLE 2

Effect of Number of Points per Standard Deviation Unit on the Accuracy and Precision of Calculating Retention and Differences in Retention[a]

σ	Peak maximum	Peak mean	Difference in maximum	Difference in mean	Difference at half height
5	118.703 ± 0.023	119.022 ± 0.022	1.001 ± 0.034	0.994 ± 0.027	1.011 ± 0.015
10	118.348 ± 0.028	118.981 ± 0.018	1.008 ± 0.029	1.010 ± 0.031	1.004 ± 0.048
15	118.011 ± 0.032	118.943 ± 0.028	1.012 ± 0.065	0.992 ± 0.044	0.991 ± 0.085
20	117.693 ± 0.025	118.904 ± 0.033	0.979 ± 0.056	0.997 ± 0.033	1.018 ± 0.061
30	116.978 ± 0.056	118.878 ± 0.043	0.984 ± 0.096	0.997 ± 0.074	0.981 ± 0.076
40	116.279 ± 0.072	119.139 ± 0.054	0.939 ± 0.089	0.948 ± 0.083	1.019 ± 0.146

[a] $\tau/\sigma = 0.5$, noise $= \pm 0.15\%$.

TABLE 3

Effect of τ/σ on the Accuracy and Precision of Calculating Retention and Differences in Retention[a]

τ	Peak maximum	Peak mean	Difference in mean	Difference in maximum	Difference on side
2	119.019 ± 0.017	119.029 ± 0.012	1.004 ± 0.024	0.997 ± 0.012	1.005 ± 0.020
6	118.610 ± 0.019	119.000 ± 0.011	1.010 ± 0.034	0.998 ± 0.019	0.999 ± 0.016
10	117.624 ± 0.020	118.970 ± 0.016	1.000 ± 0.029	1.005 ± 0.020	0.999 ± 0.013
20	113.113 ± 0.029	118.887 ± 0.023	0.998 ± 0.035	1.017 ± 0.019	1.014 ± 0.029
40	99.676 ± 0.040	118.705 ± 0.045	1.021 ± 0.046	0.974 ± 0.061	1.026 ± 0.025

[a] $\sigma = 20$ points, noise $= \pm 0.15\%$.

TABLE 4

Difference between Peak Variance as Calculated by the Second Moment and Width at Half Height for Different τ/σ Ratios[a]

τ/σ	M_2	$W_{1/2}$	$W_{1/2}^2/5.546$
0	99.966	23.56	100.04
0.1	100.89	23.660	100.93
0.3	108.88	24.436	107.66
0.5	124.84	25.588	118.06
1	199.56	28.910	150.70
2	480.07	35.860	231.86

[a] Peak has σ = 10 points.

Calculation of Peak Variances

In the calculation of peak variance, the width at half height is normally used on the assumption that the peak is Gaussian in shape. A second approach is based upon the second statistical moment. For Gaussian peaks these two measures of peak variance are equivalent; however, for skewed peaks they can be substantially different as shown in Table 4. The difference between the two calculations increased as the τ/σ ratio became larger. For a τ/σ ratio of 2, the variance calculated from the half height was only 48% of the second moment. Hence the width at half height is not very sensitive to tailing effects, with the result that calculations of theoretical plates can be seriously in error.

DISCUSSION

The present study has shown that the errors introduced in the retention time from curve fitting of skewed peaks are small and in most cases would not be significant. However, in the separation of similar species, such as isotopically substituted molecules, these differences can be significant. Fortunately, peak separation can be determined with equal accuracy and precision from the three methods. Species such as $^{12}CO_2$ and $^{13}CO_2$ have previously been fractionated in this laboratory at the natural abundance level on short packed columns using a quadrupole mass filter as a detector (*12*). There were very small differences in the retention times of 1 to 2 sec for these species, which were difficult to measure precisely. It is therefore important in some cases to obtain the best accuracy and precision in the calculation of retention and differences in retention.

Because of the low natural abundance of ^{13}C, the signal-to-noise ratio for this species was much lower than that for ^{12}C. Furthermore, the CO_2 molecules gave tailed peaks having a flat noisy maximum which made it even more difficult to calculate the peak maximum for the ^{13}C species. In that study the peak mean was not calculated because the difference in the time between the means for the two isotopic species was much smaller than the sampling interval used to collect data across the entire peak. For these reasons the peak separation was calculated at the half height on the front side of the peaks and was found to give better precision than that obtained from the maxima. The present study suggests that the better precision was fortuitous.

Although peaks are sometimes characterized with small numbers of points, the present study shows that the accuracy and precision of calculating the retention time can be improved by using a large number of points. Furthermore, one should generally use a number of points in a digital smooth which includes at least ±0.5 standard deviation units and, for higher levels of noise, ±0.75 units. In that way one can obtain the desired accuracy for small differences in retention time, especially in cases involving isotopic species where peaks will have very similar shapes.

Acknowledgments

This work was supported by the U.S. Energy Research and Development Administration through Contract E(38-1)-854. We also wish to thank the University of Georgia for a Graduate Assistantship (to R.E.P.).

REFERENCES

1. E. Grushka, M. N. Myers, P. D. Schettler, and J. C. Giddings, *Anal. Chem.*, *41*, 889 (1969).
2. E. Kucera, *J. Chromatogr.*, *19*, 237 (1965).
3. P. R. Rony and J. E. Funk, *J. Chromatogr. Sci.*, *9*, 215 (1971).
4. S. N. Chesler and S. P. Cram, *Anal. Chem.*, *43*, 1922 (1971).
5. J. C. Sternberg, *Adv. Chromatogr.*, *2*, 205 (1966).
6. L. J. Schmauch, *Anal. Chem.*, *31*, 225 (1959).
7. H. W. Johnson and F. H. Stross, *Ibid.*, *31*, 357 (1959).
8. I. G. McWilliam and H. C. Bolton, *Ibid.*, *41*, 1755 (1969).
9. H. M. Gladney, B. F. Dowden, and J. D. Swalen, *Ibid.*, *41*, 883 (1969).
10. A. H. Anderson, T. C. Gibb, and A. B. Littlewood, *J. Chromatogr. Sci.*, *8*, 640 (1970).
11. E. Grushka, *Anal. Chem.*, *44*, 1733 (1972).
12. A. T. Shepard, N. D. Danielson, R. E. Pauls, N. H. Mahle, P. J. Taylor, and L. B. Rogers, *Sep. Sci.*, *11*, 279 (1976).

13. W. W. Yau, Personal Communication, January 1976.
14. A. Savitzky and M. J. E. Golay, *Anal. Chem.*, *36*, 1627 (1964).
15. H. T. Davis, *Tables of the Mathematical Functions*, Vol. 11, Principia Press, San Antonio, Texas, 1963, p. 307.

Received by editor September 20, 1976