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Effect of noise on peak heights calculated using an exponentially modified gaussian peak shape model

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ABSTRACT

Most computer-based methods for finding chromatographic peak heights are relatively crude, relying on finding an appropriate baseline, then measuring a maximum signal height relative to the baseline. The error in finding a precise signal height of a weak signal can be increased by noise spikes. In this article, data are presented to show that the use of the exponentially modified gaussian peak shape model can effectively increase the quality of height measurements of peaks deliberately degraded to near undiscernability by dilution.

INTRODUCTION

The measurement by computer-based data systems of peak heights generated by chromatographic techniques is generally carried out by automating manual methods, *i.e.*, finding the appropriate baselines on either side of a peak and assigning the peak height by measuring the signal maximum relative to the baseline. This method can lead to imprecise data, if the signal is noisy because the real peak height maximum can be misassigned by noise spikes.

Although peak area measurements can be used, peak height measurements are generally preferred because of their better accuracy and precision [1]. Problems associated with peak area measurements include interferences with compounds eluting close to the analyte peak and uncertainty in assigning the beginning and end of a peak.

Several years ago, our automated data acquistion and processing system, QSIMPS (quantitative selected-ion monitoring processing system) was developed to quantitate drug concentrations in plasma and/or urine samples from pharmacokinetic experiments [2–4]. Because of the high sensitivity requirement of drug assays, peaks are generally fairly noisy at the lower limit of quantitation. For this reason, the exponentially modified gaussian (EMG) peak shape model [5–15] was incorporated into QSIMPS because it is widely regarded as giving the most accurate description of chromatographic peaks. Additionally, the EMG model has been shown to give results more precise than manual measurements [16].

This paper describes a study of the effect of noise on peak heights calculated using the EMG model.

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EXPERIMENTAL

The data was obtained from a gas chromatographic–mass spectrometric (GC–MS) plasma assay for rimantadine, an antiviral agent [17]. Only calibration standards and quality assurance (QA) samples were used. The calibration standards contained either 500, 200, 50, 20 or 5 ng/ml of rimantadine and 100 ng/ml of [$^2H_4$]rimantadine. The QA samples contained either 64 ng/ml (QA high) or 25 ng/ml (QA low) of rimantadine and 100 ng/ml of [$^2H_4$]rimantadine. All samples were diluted by a factor of 1000, 5000, 10 000 or 20 000 and were analyzed in duplicate. These dilution factors were chosen in order to obtain both high quality and noisy peaks at the same electron multiplier setting. All four diluted sets of calibration and QA samples were analyzed together on four separate days.

A Finnigan Model 9500 gas chromatograph was equipped with a 4 ft. × 2 mm I.D. glass column packed with 3% OV-1 on 100–120 mesh Gas-Chrom Q (Alltech). Methane (Liquid Carbonic, 99%) at 14 kg/m^2 was used as the GC carrier gas and negative chemical ionization (NCI) reagent gas. The injector, column, interface oven and transfer line were operated at 300, 250, 250 and 240°C, respectively. Prior to use, the column was conditioned with a 3-μl injection of Silyl-8® (Pierce). The GC system was equipped with an air-actuated divert valve which allowed the diversion of the solvent front from the mass spectrometer. An aliquot of 2 μl of the sample (50 μl total volume) was injected onto the column using a modified Hewlett-Packard autosampler (Model 7672A; Palo Alto, CA, U.S.A.).

A Finnigan 3200 quadrupole mass spectrometer was tuned to give the maximum response consistent with reasonable ion peak shape and near unit resolution. The voltage across the continuous dynode electron multiplier was −800 V and the voltage on the conversion dynode was +3.0 kV. The unlabelled and deuterium-labelled ions were monitored using a Finnigan Promim® (programmable multiple ion monitor, Model 015-80) unit. Each channel was set up to monitor either the $[M-HF]^{-\cdot}$ ion (m/z 353) of the unlabelled analyte or the $[M-{}^2HF]^{-\cdot}$ ion (m/z 356) of the tetradeuterated reference standard. QSIMPS [18] was used to control the autosampler, the divert valve, and to collect and process the SIM data.

The calibration curves were fit using weighted ($1/y^2$) non-linear regression, to the following equation:

$$R = \frac{P1 + x}{P2(x) + P3}$$

where R is the ion ratio [(m/z 353)/(m/z 356)], x is the analyte concentration, and $P1$, $P2$ and $P3$ are parameters adjusted to give the best fit to the calibration data.

For the signal-to-noise (S/N) calculation, noise was defined as the detector signal range between parallel lines that enclose random fluctuations for 25 scans starting with scan 125 [19], and the signal was defined as the maximum intensity at the apex of the peak minus the baseline response.

RESULTS

Fig. 1 shows SIM current profiles of the $[M-HF]^{-\cdot}$ ion (m/z 353) from rimantadine and the $[M-{}^{2}HF]^{-\cdot}$ ion (m/z 356) from $[^{2}H_4]$rimantadine from the 5 ng/ml standard diluted factors of 1000, 5000, 10 000 and 20 000. The raw data is represented by the solid line, actually made up to 512 data is represented by the solid line, actually made up to 512 data points over the retention time window shown. The crosses represent the EMG fit using the top 80% of the peak. The baseline was chosen by extrapolation between the average voltage from scans 90–100 and the average voltage from 450–460 scans. Note the good correspondence of the calculated and raw data. This figure also demonstrates the excellent sensitivity of the assay; the most dilute sample respresents 4.2 ag injected on column.

The results of all the data for the diluted calibration standards are compiled in Table I. The overall accuracy (amount found *versus* amount added) summarized in Table I can be seen to be surprisingly good. In no case is the mean found value more than 10% different than the added value. The relative standard deviations are acceptable except at the two lowest concentrations at the 1:20 000 dilution. However, for this amount of analyzed material, the noise is so high and the signal is so small, that a peak often cannot be visually discerned and no calculation of signal to noise could be calculated. The overall mean inter-assay precision can be seen to diminish more than three times, while the overall mean intra-assay precision diminished over six times, over the twenty-fold dilution range. The correlation coefficients for the fit of the calibration data to the equation used were all greater than 0.99.

Data for the high and low QA samples at the various dilutions are shown in Table II. The analyte response for all of these samples gave a discernible peak. Student's t-test analysis of the measured concentrations for each standard showed no difference among the mesured values at $p<0.01$. In spite of an approximately 25–45 fold decrease in the S/N ratio over the 20-fold dilution range, the relative standard deviations for the analyses only increased approximately 2-fold. A plot of relative

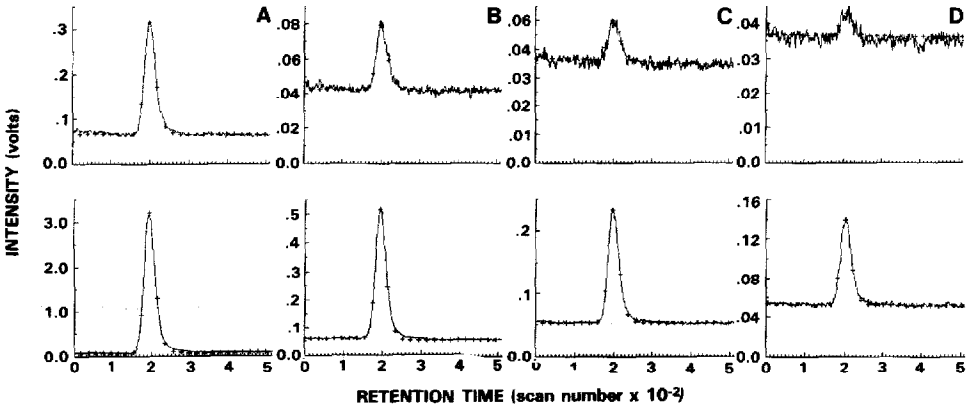

Fig. 1. SIM current profiles of m/z 353 and m/z 356 from the 5 ng/ml calibration standard diluted (A) 1:1000, (B) 1:5000, (C) 1:10 000 and (D) 1:20 000. The profile of the 1:20 000 dilution is one of the better ones at this dilution. The solid line represents the raw data and the crosses denote the EMG fit to the data.

TABLE I

CALIBRATION CURVE DATA FOR STANDARD DILUTED 1:1000; 1:5000; 1:10 000 or 1:20 000 ($n=4$).

Dilution	5 ng/ml	20 ng/ml	50 ng/ml	200 ng/ml	500 ng/ml	Inter[b]	Intra[c]
1:1000	5.0 ± 0.15 (3.0%)	20 ± 1.10 (5,4%)	52 ± 0.75 (1.5%)	196 ± 5.9 (3.0%)	505 ± 22 (4.4%)	2.8 ± 0.9%	3.0 ± 0.48%
1:5000	5.1 ± 0.40 (7.9%)	20 ± 0.64 (3.2%)	51 ± 0.64 (1.3%)	198 ± 6.6 (3.3%)	502 ± 21 (4.2%)	3.2 ± 1.3%	2.8 ± 2.4%
1:10 000	5.0 ± 0.43 (8.6%)	20 ± 1.40 (6.8%)	51 ± 0.99 (1.9%)	196 ± 3.9 (2.0%)	505 ± 13 (2.6%)	3.7 ± 1.3%	5.2 ± 3.6%
1:20 000	5.4 ± 1.30 (24%)	20 ± 2.70 (10%)	51 ± 3.00 (5.8%)	203 ± 15 (7.2%)	499 ± 16 (3.2%)	9.6 ± 2.5%	19 ± 8.0%

[a] Values between parentheses are relative standard deviations (R.S.D.).

[b] Mean R.S.D. ± S.D. for added and back-calculated concentration values.

[c] Mean R.S.D. for ratio of duplicate back-calculated concentrations (first value of duplicate pair divided y the value of the second). Calibration standards were analyzed in duplicate.

TABLE II

RESULT FOR HIGH[a] AND LOW[b] QA SAMPLES AT VARIOUS DILUTIONS

QA	Dilution	S/N[c] ± S.D.	Mean concentration[d] ± S.D. (R.S.D.)[e]
High	1:1000	272 ± 144	64 ± 2.6 (4.1%)
High	1:5000	24 ± 11	63 ± 1.9 (3.0%)
High	1:10 000	11 ± 3.2	63 ± 3.5 (5.6%)
High	1:20 000	6.4 ± 2.3	65 ± 4.3 (6.7%)
Low	1:1000	81 ± 21	25 ± 0.8 (3.4%)
Low	1:5000	11 ± 5.0	25 ± 0.7 (3.0%)
Low	1:10 000	6.0 ± 3.4	25 ± 1.4 (5.6%)
Low	1:20 000	3.0 ± 1.1	25 ± 1.8 (7.0%)

[a] QA sample containing 64 ng/ml of rimantadine from pooled patient samples.
[b] QA sample fortified to contain 25 ng/ml of rimantadine.
[c] S/N = Signal-to-noise ratio.
[d] ng/ml.
[e] R.S.D. = Relative standard deviation.

standard deviation (y) *versus* S/N (x) for the data in Table II would give a plot similar to the previously reported data of Vanderwal and Snyder [20] for high-performance liquid chromatographic drug assays. From the 1:1000 calibration data (Table I), the intrinsic precision of the GC–MS assay can be estimated to be 3%. The relative standard deviation for the high and low QA samples from the 1:5000 dilution is not too different than this intrinsic sensitivity. The relative standard deviation at a dilution of 1:10 000 is somewhat over this intrinsic precision and the relative standard deviation of the 1:20 000 dilution is considerably over the intrinsic precision.

A plot of the relative difference from global means *versus* S/N, for the high and low QA samples is shown in Fig. 2. There is a trend towards increasing error with decreasing S/N, although comparing the error in Group 8 ($S/N > 100$) to that of Group 1 ($S/N > 3$), there is only a 3-fold increase in error in spite of the >30-fold decrease in S/N.

DISCUSSION

Previously the EMG chromatographic peak model was shown to give more accurate peak height determinations than manual methods. In this study, data are presented showing that the use of the EMG model can effectively increase the quality of height measurements of peaks deliberately degraded to almost undiscernability relative to noise by dilution.

Signals from mass spectral assays are subject to all forms of noise, *e.g*, thermal, shot and exogeneous noise. Like any analytical measurement, increased sensitivity in mass spectral assays ultimately depends on incrasing the signal-to-noise ratio of the corrected response from a given amount of analyte, and decreasing the noise is a potentially effective way to accomplish this. Varous digital [21–24] methods are available to minimize noise. Analog, *i.e.*, hardware methods, are available, but can cause phase and amplitude distortion [21]. Each of the digital filtering techniques essentially uses a software algorithm to smooth and filter stored data. Some of the

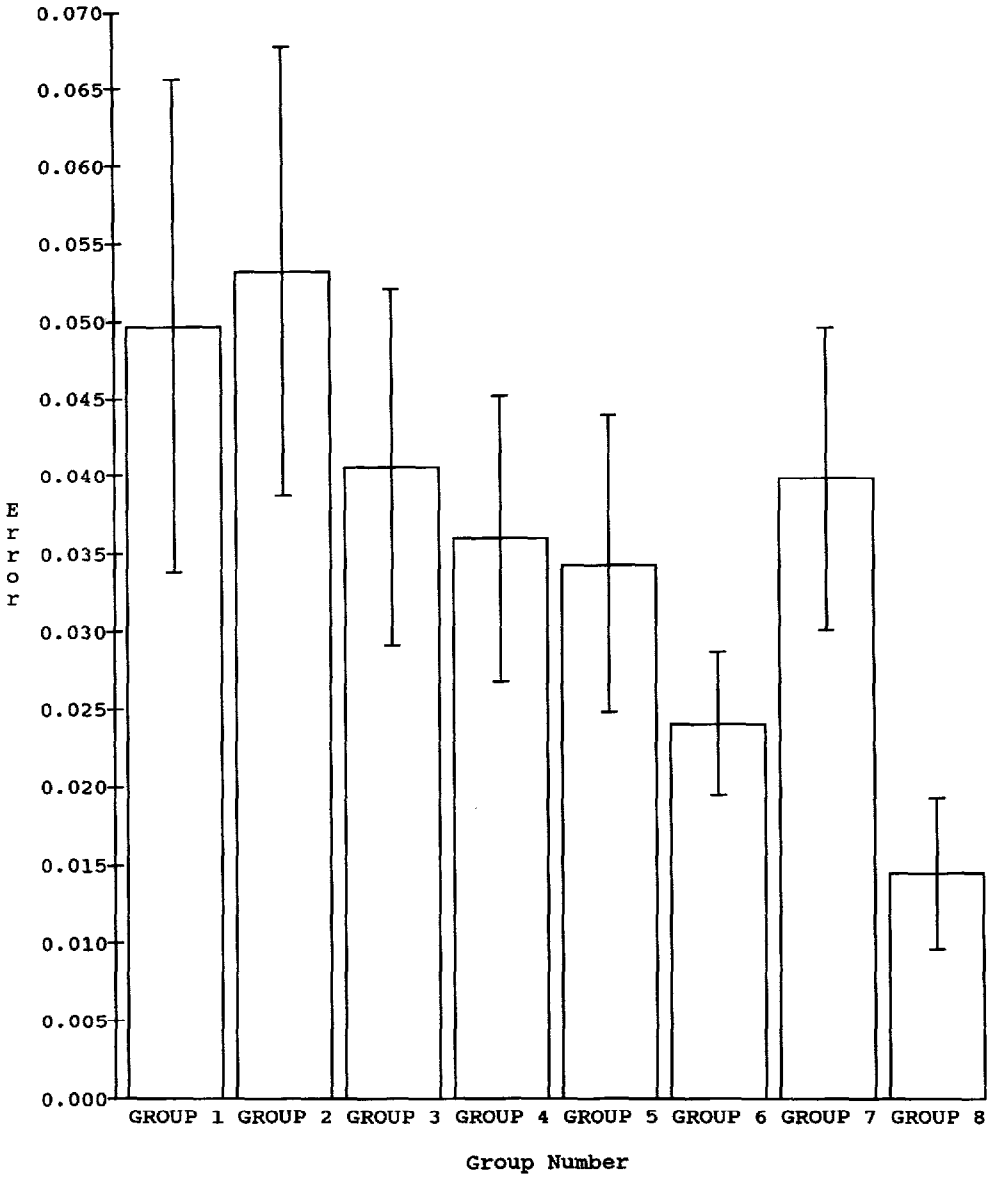

Fig. 2. Plot of relative difference (error) from global means for high and low QA samples (ordinate) *versus* S/N for the individual assay. Group 1 = S/N of 1–3 ($n=7$); Group 2 = S/N of 4 and 5 ($n=10$); Group 10 = S/N of 6–8 ($n=9$); Group 4 = S/N of 9–11 ($n=8$); Group 5 = S/N of 13–18 ($n=8$); Group 6 = S/N of 20–49 ($n=7$); Group 7 = S/N of 56–95 ($n=7$); Group 8 = S/N of 106–272 ($n=8$). All groups are not of equal size in order that no particular S/N ratio be in more than one group. Error bars are standard errors.

more common techniques are boxcar averaging [21], ensemble averaging [21], weighted or unweighted polynomial smooting [21,22] and Fourier transformation [25]. With modern microprocessors the previous disadvantage of slow processing times with complex algorithm is typically not significant.

Because no systemic direct comparison between the various smoothing routine has been done, it is difficult to evaluate whether the aproach described in this study is superior or even equal to any or all of the other methods. However, it is fundamentally different than from the others because the data are fit to a model, and not smoothed. Noise rejection in this approach is based on observed inconsistency with the model which is typically accepted as being the most accurate representation of chromatographic peaks, and not on datapoints inconsistencies with adjacent data points. The approach described in this study offers a method for rejecting noise which is based on a chromatographic, and not an electronic perspective. This is in addition to its ability to generate accurate chromatographic features of merit such as a value for theoretical plates [12,14].

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