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Note

Comparison of manual and exponentially modified gaussian based methods for the determination of the peak heights of selected-ion current profiles acquired in a mass spectral drug assay

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The exponentially modified gaussian (EMG) peak shape model[1–14] is widely regarded as giving the most accurate description of chromatographic peaks[15–17]. In particular, this model provides accurate values for chromatographic features of merit such as theoretical plates, asymmetry factors, etc.[10,11]. Surprisingly, no study has been reported on the precision of the model for calculating chromatographic peak heights.

The choice of using peak heights rather than peak areas for quantitative selected-ion monitoring (SIM) measurements has been made over the course of several years' experience with many varied drug assays by ourselves and others[18–20]. The accuracy and precision of peak height measurements exceeds that of peak area calculations, due primarily to interfering compounds eluting close to the analyte of interest and problems in assigning the beginning and end of a peak. The use of peak areas usually requires baseline separation, whereas poorer separations can be tolerated by peak height measurements.

Several years ago, the use of the EMG model to calculate peak heights was incorporated into quantitative selected-ion monitoring processing system (QSIMPS), a collection of hardware and software for the automated collection and analysis of SIM data acquired for use in pharmacokinetic studies[21–23]. First, an estimation of the peak height is done by utilizing a user-chosen baseline along with a quadratic fit of intensity data at the top of the peak. The peak height and retention time estimated from the quadratic fit are then used as initial parameters for fitting to the EMG equation. Briefly, QSIMPS uses NONLIN[24], a popular non-linear regression program, to fit the baseline subtracted ion intensity-time data to the EMG equation shown below:

$$h(t) = \frac{AS}{T}\left(\frac{\pi}{2}\right)^{1/2} \exp\left[\left(\frac{S}{T}\right)^2 \frac{1}{2} - \frac{t-R}{T}\right] \int_{-\infty}^{Z2^{\frac{1}{2}}} \frac{1}{(2\pi)^{1/2}} \exp\frac{-y^2}{2}\,dy$$

where $h(t)$ is he peak height at time t, A is the gaussian peak amplitude, S is the standard deviation of the gaussian distribution, T is the time constant of the exponential decay, R is the center of gravity (retention time) of the gaussian peak, and

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$$Z = 2^{-1/2}\left[\frac{(t - R)}{S} - \frac{S}{T}\right]$$

The resulting (returned) values for A, S and R are then used with the time values to calculate a maximum value for the intensity of the peak (peak height). This process is followed for both the analyte peak and the internal standard peak, and the ratio of analyte to internal standard is calculated. The ratio measured in an experimental sample is converted to an amount of analyte using calibration data obtained from the analysis of calibration standards containing various amounts of analyte and a fixed amount of internal standard.

In this note, peak heights of selected ion current profiles determined using the EMG based method used in QSIMPS, are compared with heights determined by manual measurements.

EXPERIMENTAL

The comparison was based on data from a gas chromatographic–mass spectrometric plasma assay for rimantadine[25], an antiviral agent. In this assay, quality assurance samples and calibration standards containing either 500, 200, 50, 20 or 5 ng ml^{-1} of rimantadine were analyzed in duplicate. All samples were fortified with 100 ng ml^{-1} of tetradeuterated rimantadine.

A Carlo Erba gas chromatograph was equipped with a capillary column, Chrompack® CP-Sil 8 CB (25 m × 0.33 mm I.D., film thickness 1.25 μm). The column was maintained at 265°C with methane as the carrier gas. The flow was set to give $1 \cdot 10^{-4}$ Torr source ion gauge reading. The injector, column and interface/transfer line were set to 300, 265 and 300°C, respectively. Under these conditions the retention time of rimantadine was 2.8 min. A Hewlett-Packard Model 7672A automatic liquid sampler was used to inject samples. This auto sampler has a sample capacity of 99 samples.

A Kratos MS-50 magnetic sector mass spectrometer was tuned to give the maximum response consistent with reasonable ion peak shape and a resolution of about 7000. Methane was used as the negative chemical ionization reagent gas. The unlabelled and deuterium-labelled ions were monitored by a Vacuum Generators (VG) digital multiple ion detection (DIGMID) system wired to control the MS 50. These ions were monitored relative to an external lock mass of $C_7F_{14}^-$ (m/z 350) from perfluorotributylamine. The actual ions monitored were the $[M-HF]^-$ ion (m/z 353) of the unlabelled analyte, and the $[M-{}^2HF]^-$ ion (m/z 356) of the tetradeuterated-labelled internal standard.

The output of the DIGMID was sent both to a Lenseis (Princeton Junction, NJ, U.S.A.) eight channel recorder and to QSIMPS. Several recorder channels, set at various attenuations, were connected together so that measurable peaks were obtained for both analyte and internal standard regardless of the response from the sample. The essential components of the QSIMPS hardware consisted of a Hewlett-Packard HP-1000 (A900 processor) with 4.5 Mb of memory, a Model 7937 disk drive with 571 Mb of memory, three HP 3497A interfaces, four HP-2623A terminals, an HP2608S printer with graphics capability, and HP9144A streaming cartridge tape units.

A total of eighteen sets of calibration samples (five calibration concentrations and one quality assurance sample, all analyzed in duplicate) were assayed over a period of several months. The heights of the peaks on the chart paper output of the recorder were measured with a ruler to a resolution of one millimeter. The measured values were manually entered into QSIMPS and were compared with those obtained using the EMG model. Both the manually measured and EMG calculated peak heights for the calibration standards were then regressed against the concentration values. The parameters from the regression analyses were then used to calculate values for the quality assurance sample. The inter-assay precision was estimated from the difference between the observed value for a concentration and the concentration back-calculated from the regression line. The intra-assay precision was estimated from the ratio of the duplicate analyses.

RESULTS AND DISCUSSION

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. The solid line represents the raw data, actually made up of 512 data points over the retention time window shown. The crosses represent the EMG fit using the top 95% of the peak. The baseline was chosen by extrapolation between the average voltage from scans 150–160 and scans 400–410. Note that the calculated peak height and the peak height from the SIM chromatogram are nearly identical. Also, since only the top 95% of the peak was used for the fit, note that the calculated peak tail is slightly less than the actual SIM profile.

Data from the comparison are given in Table I. Both methods of peak height measurement gave similar results, *i.e.*, a linear regression analysis of back-calculated concentrations measured either by hand (y) or by the EMG-based method (x) gave a slope of 1.006 and an intercept of 0.76 ng ml^{-1}. The correlation coefficient for the regression was 0.9999.

Almost without exception, the comparison of the data from both methods of peak height measurement showed that the EMG based method gave superior results. Compared with the manual measurements, the inter-assay precision from the EMG based measurements was lower at every calibration concentration and for the quality assurance sample. The mean improvement in relative standard deviation was 16%. Compared with the manual measurements, the intra-assay precision from the EMG based measurements was lower at three of the five calibration concentrations and for the quality assurance sample. The mean improvement (including the two concentrations not showing improvement) was approximately 5%.

The EMG model for chromatographic peaks has not previously been incorporated into an on-line data system. This model is valued by professional chromatographers because it accurately accounts for the internal and extracolumn processes responsible for peak tailing. However, to the ordinary chromatographer, the essential citerion for any chromatographic peak model is whether it improves the precision of the collected data. In this regard, SIM current profiles are a good test of any model because of the high degree of noise associated with such measurements. The results reported here suggest that the EMG model yields a more precise determination of the peak heights than hand measurements. In addition, it should be noted that, although

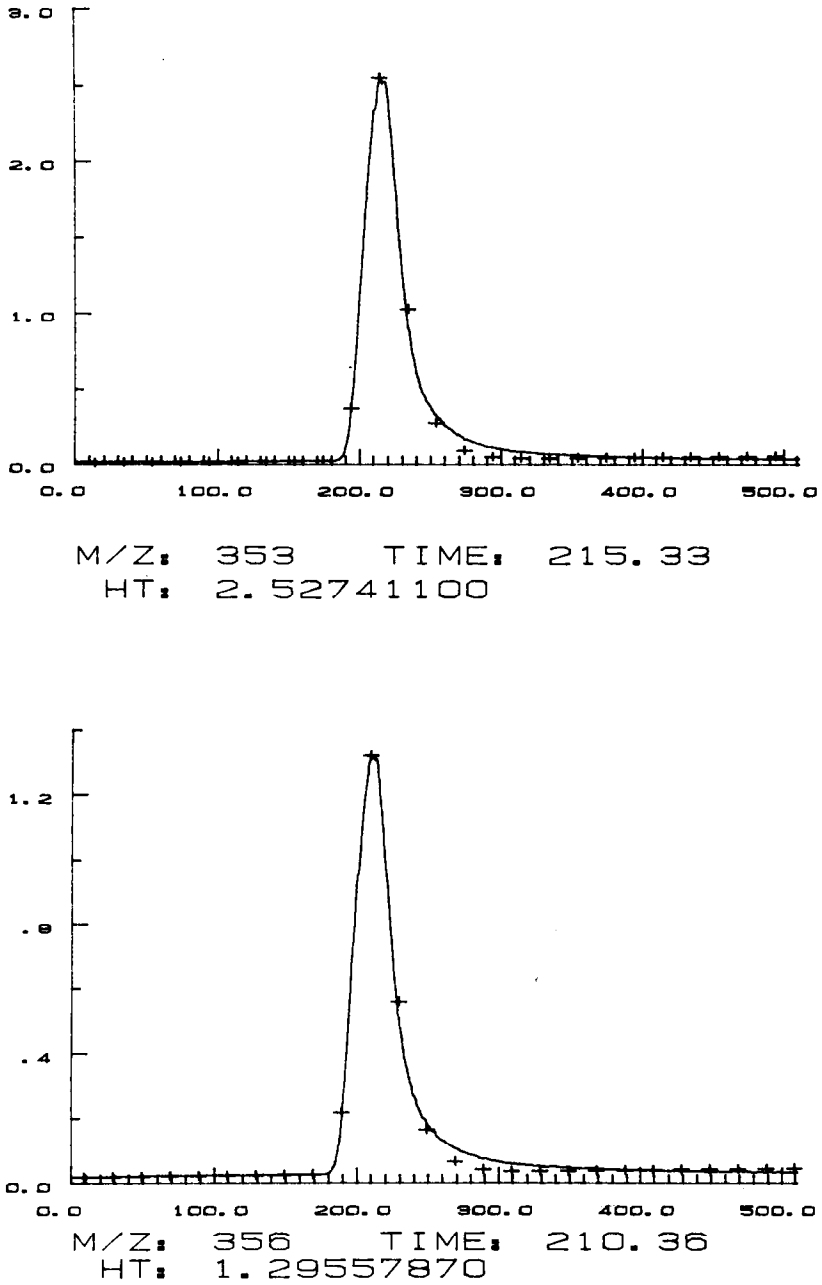

Fig. 1. Selected-ion monitoring current profiles of the $[M-HF]^{-\cdot}$ ion (m/z 353) from rimantadine and the $[M-{}^2HF]^{-\cdot}$ ion (m/z 356) from [$^2H_4$]rimantadine. The raw data is represented by the solid line and the crosses represented the calculated EMG fit using the top 95% of the peak.

TABLE I

COMPARISONS OF DATA FROM THE ANALYSIS OF THE SAME SAMPLE WITH PEAK HEIGHTS DETERMINED BY MANUAL AND EMG-BASED METHODS

Data were collected and processed as described in the Experimental section. Data in parentheses are for the EMG-based measurements. Data from manual measurements are without parentheses.

Sample[a]	*Inter-assay precision (found concentration ± S.D.*[b]*, R.S.D.*[c]*)*	*Intra-assay precision (ratio*[d] *± S.D., R.S.D.)*
500 ng/ml	496 ± 12, 2.3% (492 ± 9.0, 1.8%)	0.98 ± 0.06, 6.1% (0.98 ± 0.04, 4.1%)
200 ng/ml	202 ± 12, 5.8% (206 ± 9.0, 4.4%)	0.99 ± 0.06, 6.0% (1.01 ± 0.06, 6.0%)
50 ng/ml	53 ± 3.9, 7.4% (54 ± 3.9, 7.2%)	1.00 ± 0.04, 4.0% (1.00 ± 0.04, 4.0%)
20 ng/ml	21 ± 2.0, 9.2% (21 ± 1.5, 7.1%)	1.05 ± 0.11, 10% (1.02 ± 0.09, 9.0%)
5 ng/ml	4.3 ± 0.9, 21% (4.3 ± 0.8, 19%)	1.00 ± 0.22, 22% (0.97 ± 0.25, 26%)
Quality assurance	63 ± 5.5, 8.7% (63 ± 3.1, 5.0%)	1.00 ± 0.09, 9% (1.01 ± 0.05, 5.0%)

[a] Eighteen calibration curves (five calibration standards and one quality assurance sample, all analyzed in duplicate).

[b] S.D. = Standard deviation.

[c] R.S.D. = Relative standard deviation.

[d] Ratio of back-calculated concentration from first determination of the duplicate pair divided by back-calculated concentration from second determination.

the SIM current profiles used in this comparison had excellent signal-to-noise characteristics, the chromatographic conditions varied significantly over the course of the experiment, demonstrating that the EMG model can accurately describe both sharp and tailing peaks.

Most mass spectrometer data systems determine peak heights of SIM current profiles by essentially automating conventional manual methods, *i.e.*, finding an appropriate baseline and peak, and assigning a peak height by finding the maximum voltage for the peak. This process can easily lead to inaccurate results when the peaks have low signal-to-noise ratios because the data system will often assign a voltage maximum which corresponds to a noise spike. Although, these noise spikes and other irregularities can be easily recognized visually, peak height determinations based on manual measurements were still found to be less precise then EMG based measurements.

Model based peak height measurements offer potential advantages in chromatographic assays. When the analyte being measured is known, the parameters describing the shape of the analyte peak in an experimental sample can be compared with the parameters from a stable isotope internal standard in order to verify compound identification. For example when an electronic noise spike occurs in the retention time window of interest, it is desirable that the peak be disregarded. One way to do this is to compare the peak fitting parameters of the stable isotope reference standard to that of the noise spike. In most cases, the peak width of the noise spike is different than that of the authentic standard and therefore the spike would not be identified as the analyte peak. This capability is not currently being applied in our laboratory but is a possible application of model based peak height measurements. Model based characterizations are a starting point for the greater use of the increased

computational capacity now available to an analyst to interpret and characterize chromatographic data. In this regard, the EMG model is still a relatively unrefined representation of the phenomena occurring in a chromatographic column. The EMG model will certainly be succeeded by more accurate expressions which, based on the results reported here, should yield even more precise peak height measurements.

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