DOES PEAK DECONVOLUTION RESULT IN MORE ACCURATE AND PRECISE ENANTIOMER FRACTIONS FOR CHIRAL ENVIRONMENTAL CONTAMINANTS?

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Introduction

Many chemicals of environmental interest are chiral, and exist as pairs of enantiomers. The enantiomer composition of chiral compounds can provided increased insights into biological processes affecting these chemicals. Physical and chemical environmental processes are generally identical for such stereoisomers. However, individual enantiomers can have very different biological and toxicological effects due to differential interactions with other chiral molecules (e.g., enzymes in living organisms). Thus, enantioselective environmental analysis is a powerful tracer of biogeochemical environmental processes. Such quantification is typically done by means of enantioselective analytical separations such as gas chromatography (GC) or high performance liquid chromatography (HPLC), and is usually quantified by means of the enantiomer fraction[1]:

$$EF = \frac{(+)}{(+)+(-)} or \frac{E1}{E1+E2} \quad (1)$$

where (+) and (-) are the concentrations of the (+)- and (-)-enantiomers, respectively, and E1 and E2 are the respective concentrations of the first- and second-eluted enantiomers using specific analytical conditions, when optical rotation order is not known.

The EF values from environmental samples can be applied to gain a better understanding of the environmental fate of chiral chemicals. For example, the EFs of polychlorinated biphenyls (PCBs) in the Hudson River estuary have been used to determine the extent of riverine vs. atmospheric sources to the estuary for remediation purposes[2]. Measurements of EF have also been used to determine enantioselective rates of biotransformation in organisms, such as fish, that degrade persistent organic pollutants slowly enough that non-enantioselective methods would be incapable of measuring them[3,4]. However, calculations involving EF may be sensitive to slight errors in EF, particularly for enantiomers that are only partially resolved as is common in enantioselective environmental analysis[5]. Thus, accurate determination of EF is important.

There are several methods for integrating partially resolved peaks from enantioselective chromatographic separations, including the valley drop method (VDM) and the deconvolution method (DM). The VDM involves dropping a vertical line in the centre of the valley between two peaks and integrating the peak areas on either side of that line. This method is the most widely used and is performed with standard chromatographic integrators. The DM involves least squares fitting of the chromatogram to two mathematical functions that mimic chromatographic peak shapes. The area beneath each of the deconvoluted peak functions is then calculated. This method has been used by some workers in environmental enantiomer analysis[3,4,6,7]. However, it has not been commonly adopted. While studies have evaluated different integration methods[8] and associated error[9], no previous study to our knowledge has investigated the effect of such error on EF, nor on calculations based on it.

The objective of this study was to determine the relative accuracy and precision of EFs calculated using these two integration methods using simulated chromatographic data. The simulated chromatographic data approach allows for a high level of control over peak parameters that could not be achieved with real chromatograms. This control allows for the investigation of the effects of individual variables on EFs calculated using the two integration methods. Simulated data have previously been used to investigate other problems in chromatographic data handling. Applications of simulated chromatograms include comparing the effects of

modulation ratio and retention time shifts on the precision and accuracy of comprehensive two-dimensional gas chromatography (GC×GC) data when using multivariate and summed-peak approaches to quantifying data[10].

Methods and Materials

Simulated chromatographic data was created using MathCAD 14.0 (Parametric Technology, Needham, MA, USA) software. The simulation utilized the 4 parameter exponentially modified Gaussian—Half Gaussian Modified Gaussian hybrid (GEMG4) function[11] to simulate chromatographic peak shapes, as has been previously suggested[7]. The sum of two GEMG4 functions was used to generate a chromatogram simulating the results of an enantioselective separation, and was solved at intervals of 1/94 minutes to simulate a data acquisition rate of 1.57 Hz. To mimic the noise found in actual chromatographic data, random numbers with a Gaussian distribution and a chosen standard deviation, σ_N, were added to the sum of the GEMG4 functions at each acquisition time. The numbers had an average value of 150 in order to provide a baseline above zero and avoid problems with the integration software due to negative values. The simulated chromatograms were then exported as tab delimited text files, and time markers added via spreadsheet before conversion of the text files to chromatographic CDF format using GC File Transfer Pro 5.0 (ChemSW, Fairfield, CA, USA) software. Simulated chromatograms were created with specific combinations of EF, resolution, peak asymmetry, and signal to noise ratio (S/N). The EF was controlled by adjusting the areas of each of the two GEMG4 functions. The area values corresponding to each desired EF were calculated using (1). The remaining parameters were calculated within the MathCAD worksheet using the individual GEMG4 functions before summation and addition of noise. The chromatographic resolution, R_s, was calculated:

$$R_s = \frac{2(t_{rb} - t_{ra})}{W_{ba} - W_{bb}} \tag{2}$$

where t_{ra} and t_{rb} are the respective retention times of the first-eluted and second-eluted peaks and W_{ba} and W_{bb} the base widths of these two peaks. The peak width at the base of each peak was calculated at one percent of the maximum peak height. The peak asymmetry, A_s, was calculated:

$$A_s = \frac{W_r}{W_t} \tag{3}$$

where W_r is the width of the right half of a peak and W_l is the width of the left half of a peak, both calculated at ten percent of the maximum peak height. The appropriate σ_N to generate a desired signal-to-noise ratio (S/N) was determined by dividing the maximum value of the GEMG4 function for the largest peak by the desired S/N ratio. A set of ten simulated chromatograms, each with a different set of numbers but the same σ_N added as noise, was generated for selected combinations of the following parameters: A_s = 0, 1.5, 2.5, and 3.5; R_s from 0.5 to 1.5 in intervals of 0.2; S/N = 10, 20, and 75; and EFs from 0.2, 0.4, 0.45, 0.5, 0.55, 0.6, and 0.8.

Each simulated chromatogram was integrated twice, once using the VDM and once using the DM. The VDM integrations were carried out using the MSD Chemstation Integrator (version E.01.00.237; Agilent Technologies, Mississauga, Canada) and the CDF format data files. Boundaries for integrating the peaks were selected to include a division between peaks at the centre of the valley, and areas given by the integrator for the peaks were used to calculate the EF given by the VDM. The DM integrations were carried out using PeakFit 4.0 (SPSS Inc., Chicago, IL, USA) software to fit the simulated chromatograms in text format to two GEMG4 functions. Software options to use a least squares fitting and to vary both the shape and width of the peaks were selected. The fit was adjusted until the r^2 value reached a maximum. The peak areas given by PeakFit were then used to calculate the EF given by the DM. For each set of ten chromatograms with identical parameters, the average bias in EF was calculated for each method as follows:

$$\text{average bias} = \frac{\sum_1^n EF_n}{n} - EF_{actual} \tag{4}$$

where *n* is the number of identical chromatograms in the trial, EF_n the EF determined by a particular integration method for the nth chromatogram, and EF_{actual} is the actual EF being simulated. The standard deviation of EF_n determined using each integration method was also calculated giving the standard deviation of EF (σ_{EF}).

Results and Discussion

Through integration of a large number of simulated chromatograms, we found that the VDM has larger biases in EF associated with it than the DM does. For asymmetric peaks with EFs between 0.2 and 0.8 and resolutions between 1.5 and 0.5, the VDM had average biases between -0.058 and +0.052 (Figure 1), while the DM had average biases between -0.014 and 0.017 (Figure 2). While the average biases in EF with the DM had no apparent pattern with changes in resolution or EF, the VDM average biases followed a couple of patterns. The average bias in EF with the VDM tends to become increasingly negative with increasing EF. In addition, for resolutions of 0.9 or less, the VDM tends to give increasingly negative average biases in EF with decreasing resolution. With symmetric peaks, it appears that VDM average biases are smaller than with asymmetric peaks. However, with symmetric peaks the average biases may still become increasingly negative with increasing EF. Increasing the peak asymmetry beyond 1.5 appears to have little effect on general patterns of bias in EF. Average bias in EF with the DM remains small for peaks of greater asymmetry and average bias is larger with the VDM and becomes increasingly negative with increasing EF. Finally, utilizing peaks with a larger or smaller S/N ratio does not change the observed patterns in average bias. The DM is more accurate than the VDM for integrating chromatographic peaks from enantioselective separations to determine EF

The standard deviations in EFs (σ_{EF}) obtained with the two integration methods were also compared to gain an understanding of their precision. With the VDM, σ_{EF} varies little with changes in resolution and EF. For peaks of asymmetry 1.5 and a S/N ratio of 20 for the largest peak, σ_{EF} was on average 0.016 with the VDM. With the DM, σ_{EF} was much larger for peaks with a resolution of 0.5 than for peaks with greater resolutions (Figure 3). In addition, EFs further from 0.5 also led to larger a σ_{EF}. For both methods, σ_{EF} decreases with increasing S/N ratio. This effect is most pronounced for the DM. However, it should be noted that at this chromatographic resolution, peaks are barely resolved. For asymmetric peaks, it is nearly impossible to determine a valley between peaks for which to use VDM unless it is already known ahead of time, as with our simulated data. This analysis shows that even with poorly resolved peaks, DM can provide some useful information on EFs.

The DM is clearly superior to the VDM for integrating chromatographic data to determine EF due to the smaller biases in EF that result. Environmental caluculations that use EF for purposes such as source apportionment are very sensitive to slight variations in EF. Therefore, the biases observed with the VDM of between -0.058 and +0.052 would result in noticeable errors in EF-based environmental calculations if EFs with errors of these magnitude were used.

References

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Figure 1: Average bias vs. EF for the VDM in the set of chromatograms with peak asymmetries of 1.5 and S/N ratio of 20 for the largest peak (n=10).

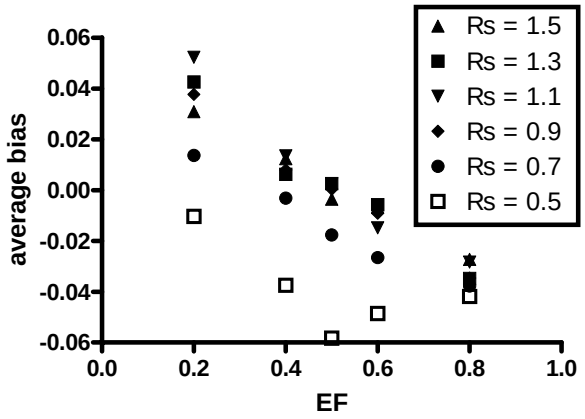

Figure 2: Average bias vs. EF for the DM in the set of chromatograms with peak asymmetries of 1.5 and S/N ratio of 20 for the largest peak (n=10).

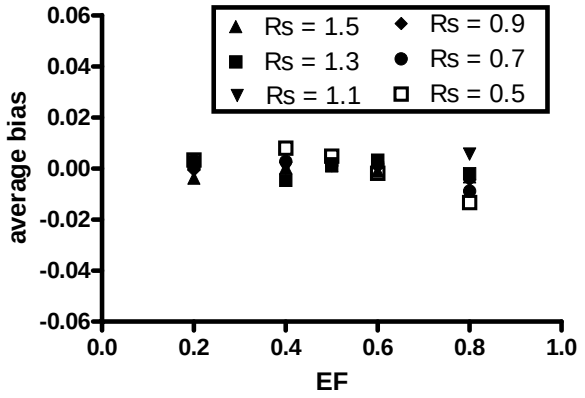

Figure 3: σ_{EF} vs. EF for both DM and the VDM at resolution 0.5 at various S/N ratios for the largest peak.