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# Comparison of peak integration methods for the determination of enantiomeric fraction in environmental samples

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ABSTRACT

Enantiomeric fractions (EFs) are used extensively in environmental pollutant research because of the insights on biochemical weathering available from quantifying enantiomeric composition. While this analysis is powerful, it can also be subject to significant error, depending on how chromatographic peaks are integrated. Two methods of integration, the common valley drop method (VDM) and the deconvolution method (DM) were compared using both instrumental and simulated chromatograms to assess their performance when integrating pairs of enantiomers. The effect of peak parameters such as true EF, peak resolution, signal-to-noise ratio, and asymmetry were also investigated. The VDM biased EFs by up to +6% to −4% (relative to the 0–1 EF scale) for symmetric peaks, and as low as −20% for asymmetric peaks. For both instrumental and simulated data, biases tended to increase with decreasing resolution and more extreme (nonracemic) EFs. In contrast, the DM produced biases that were less than 1% in most cases, including at very low resolutions. Estimates from previously published studies based on EF, such as biotransformation rate and source apportionment, could be dramatically affected by small errors in EF. Our results suggest that a deconvolution-based integration method is preferable for the handling of enantiomer compositions. Caution is also advised when comparing published studies on chiral environmental pollutants as most do not specify how chromatographic data is processed.



## 1. Introduction

The measurement of individual enantiomers of environmental contaminants is a current area of significant interest. Numerous compounds of environmental concern are chiral, including organochlorine pesticides such as α-hexachlorocyclohexane, 19 of the 209 polychlorinated biphenyl (PCB) congeners, hexabromocyclododecanes, and many pharmaceuticals such as propranolol and fluoxetine. Enantioselective analysis of a chiral compound can provide valuable information about its environmental fate and biochemical weathering (Bordajandi et al., 2008; Wong et al., 2009), including the occurrence and extent of biotransformation (Wong et al., 2004; Warner et al., 2005) and the proportions of contaminant originating from multiple sources (Ridal et al., 1997; Bidleman and Falconer, 1999; Asher et al., 2007). This also has potential implications for ecological risk assessments given the differential toxicities of the enantiomers of many chiral environmental contaminants (Hühnerfuss et al., 1995; Stanley et al., 2007; Jin et al., 2008; Wilson et al., 2008). The preferred metric for quantifying these relative concentrations is the enantiomeric fraction (EF) (Harner et al., 2000), defined as:

$$\mathrm{EF} = \frac{A}{A+B} \tag{1}$$

where $A$ and $B$ represent concentrations of the (+) and (−) enantiomers, respectively, or of the first- and second-eluted enantiomers under defined enantioselective chromatographic conditions if the elution order is unknown. Pure enantiomers have EFs of 0 or 1, while racemates have an EF of 0.5 (Harner et al., 2000). EFs are commonly used in environmental calculations when performing source apportionment (Harner et al., 2000) and when calculating minimum biotransformation rate constants (Wong et al., 2002). These calculations are sensitive to slight errors in EF. Consequently, the accuracy in determining enantiomer peak areas is especially important.

While the complete chromatographic separation of enantiomers is desirable; in practice, the quantification of environmental chiral contaminants is often performed when the two enantiomers are only partially resolved. Complete separation of enantiomers is often impractical for routine analyses, such as those quantifying several pairs of enantiomers at once (Wong and Garrison, 2000; Janák et al., 2005). The most commonly used technique for integrating partially resolved chromatographic peaks of environmental analytes is the *valley drop method* (VDM). In this process, which can

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be performed using standard chromatographic software, a perpendicular line is dropped from the valley between the two peaks to the baseline (Fig. 1). This method will always result in biased peak areas (Westerberg, 1969), except when the peaks are equal in size (EF = 0.5) and symmetric (Meyer, 1995a). When these conditions are not met, a significant portion of the area of one enantiomer's peak will inevitably fall under the peak of its antipode in disproportionate amounts (Fig. 1). Enantioselective chromatography, which often suffers from slower mass transfer kinetics and more frequent non-linear isotherms (Fornstedt et al., 1996a,b), can result in more severe peak tailing, causing even larger biases when using the VDM.

Biases associated with the VDM have been previously studied by Meyer (1995b), who showed that errors in area can be as high as 40% when working with pairs of peaks having appreciably different sizes (area ratios of 10:1) and significant tailing (asymmetry of 2). Bicking (2006) studied four different integration techniques, including the VDM and a "Gaussian skim" method, where true peak areas are estimated by adding a skimming line that approximates a Gaussian function under each peak, and adding the area between the skim line and the baseline to the parent peak. In that study, the Gaussian skim method produced errors that, in most cases, were similar to, or even worse than the VDM. A less commonly used but potentially more accurate integration technique is the *deconvolution method* (DM). Here, a least squares method is used to fit the chromatographic data to the sum of two independent Gaussian-based mathematical functions via commercially available software. Since each peak is fit to its own function, the algorithms account for peak overlap (including tailing when appropriate models are used). This results in peak areas that are not subject to the biases of the VDM. Peak deconvolution has been used successfully in the determination of environmental contaminants, including polybrominated diphenyl ether congeners (Mydlová et al., 2007), pesticides (Krupčík et al., 2005), and their enantiomerization energy barriers (Krupčík et al., 2000), and an automated deconvolution method has been developed (Shackman et al., 2004). This analysis has also been applied to comprehensive two-dimensional gas chromatographic (GC × GC) data (Kong et al., 2005).

Although the variability in error associated with traditional integration techniques has been established, details associated with peak integration have been absent from the experimental sections of chiral environmental literature, with a few exceptions (Ulrich and Hites, 1998; Asher et al., 2007; Ross et al., 2008). The potential improvement in the accuracy of enantioselective environmental analyses by using an advanced integration technique, such as the DM, has not yet been assessed. Our objective is to compare the errors in EF determination between the VDM and the DM, utilizing commercially available software for both techniques. Both instrument-generated (hereafter referred to as "real") and simulated chromatograms were analyzed to assess the accuracy and precision of each integration method, and to investigate the effects of true EF, signal-to-noise ratio, resolution, and peak asymmetry on the performance of each technique. The implications of such errors (having magnitudes observed in this study) on environmental calculations that utilize EF, using published environmental data, is also discussed.

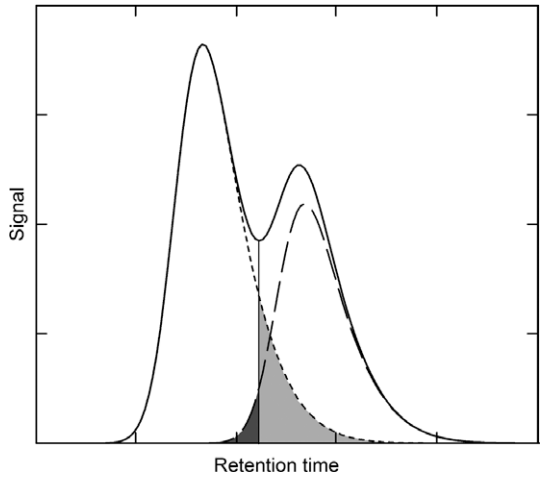


**Fig. 1.** Integration of partially resolved enantiomers by the valley drop method. Dashed lines indicate the peak traces of individual enantiomers. Shaded regions indicate the peak area of each enantiomer that is erroneously attributed to its antipode, resulting in a calculated EF that is too small. The example shown has a true EF = 0.6, $R_s$ = 1.0, and $A_s$ = 1.5.

## 2. Materials and methods

### 2.1. Preparation of enantiomerically enriched standards

PCB 132 (Fig. 2, inset) was chosen as a model compound for the real chromatograms because its enantiomers can be readily separated and collected by high performance liquid chromatography (HPLC), and can be baseline-resolved by gas chromatography (GC) (Haglund and Wiberg, 1996), providing a means for establishing a true EF. A method for isolation of individual PCB 132 enantiomers has been previously published (Haglund, 1996). Briefly, seven 50-μL aliquots of 15 μg $mL^{-1}$ racemic PCB 132 were injected into an Agilent HPLC 1050 system with a Nucleodex β-PM column (200 mm × 4.6 mm i.d. × 5 μm particle size, Macherey-Nagel, Düren, Germany). A flow rate of 0.5 mL $min^{-1}$ and a 75:25 methanol:water isocratic mobile phase was used. The eluent fractions containing individual enantiomers were collected, combined, transferred to hexane via liquid–liquid extraction, and evaporated to approximately 1 mL under nitrogen. Solutions with an approximate EF = 0.3, 0.4, 0.6, and 0.7 were generated by combining the enantiomerically pure solutions in appropriate proportions.

### 2.2. Chromatographic conditions for instrument-generated data

Analysis was performed with a HP 5890/5971 GC/MS system using electron impact ionization in selective ion monitoring mode for *m/z* of 358, 360, and 362. A Chirasil-Dex column (30 m × 0.25 mm i.d. × 0.25 μm df, Varian, Walnut Creek, CA) was used for the separation. Seven chromatographic resolutions

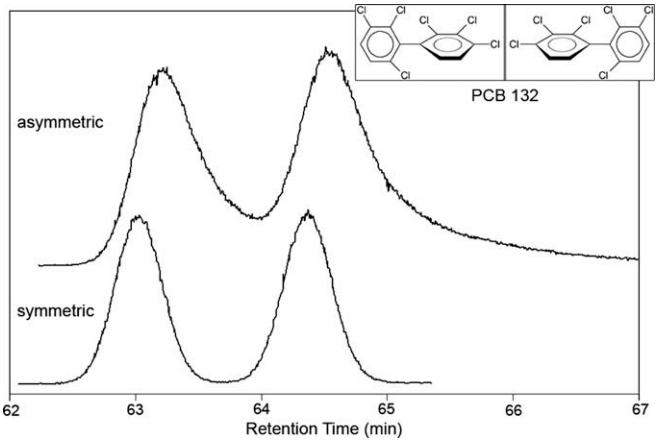


**Fig. 2.** Sample chromatograms of racemic PCB 132 standard under symmetric ($A_s$ = 1.0) and asymmetric ($A_s$ = 2.7) separation conditions. Inset: chemical structures of PCB 132 atropisomers.