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Enantiomer fractions instead of enantiomer ratios

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Abstract

The use of enantiomer ratios (ERs) to indicate the relative amounts of a pair of enantiomers in a sample has some disadvantages. Enantiomer fractions (EFs) are proposed as an alternative expression to eliminate the difficulties. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Bioaccumulation and metabolism in biota are often different for enantiomers; therefore, a change of the relative amounts in which an enantiomeric pair is present can occur during disposition in the food chain. A significant deviation from the ratio in which the enantiomers are present in the technical or commercial mixture, in which they are usually present in equal amounts, suggests a specific metabolic transformation of one of the enantiomers. A constant ratio, on the other hand, points to biological persistence or a non-specific metabolic transformation. An aspect of additional interest is that enantiomers often have different toxic properties.

Usually, enantiomer ratios (ERs) are expressed as the peak area or peak height of the (+)-enantiomer divided by that of the (−)-enantiomer (Mossner et al., 1992; Muller et al., 1992; Oehme et al., 1994; Glausch et al., 1996). When it is not known which conformation the enantiomers eluting from a chromatographic column have, ER is often expressed as the peak area or height of the first eluting enantiomer divided by that of the second one (Kallenborn et al., 1994)

$$\mathrm{ER} = \frac{\text{Peak area of enantiomer 1}}{\text{Peak area of enantiomer 2}}. \quad (1)$$

Other expressions used are the enantiomeric excess (e.e.) and the chromatographic purity (c.p.) (Bicchi et al., 1994; Beesley and Scott, 1998)

$$\text{e.e.} = \frac{R-S}{R+S} \times 100\%, \quad (2)$$

$$\text{c.p.} = \frac{R}{R+S} \times 100\%, \quad (3)$$

where R and S are the well-known indications for the structural conformation of the enantiomers.

In the daily practice, ER is the parameter most frequently used. However, as will be outlined in Section 2, its use has some disadvantages and an alternative expression will therefore be proposed.

2. Discussion

Calculating the ratio of two enantiomers by means of ER gives an undefined result when the second enantiomer is not, or cannot be, detected. This was observed in

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a previous study (de Geus et al., 1998) and, therefore, the peak area of the second enantiomer was divided by that of the first one instead (ER′). Of course, this approach only shifts, and does not solve, the problem. The proper way to solve this problem is to divide the peak area of interest by the limit of detection expressed as peak area (which does not equal zero) of the enantiomer which is not found.

The second problem connected with the use of ERs, is that plots of ER versus the peak area of enantiomer 1 are not linear (Fig. 1). This makes comparison of enantiomer ratios somewhat difficult because ER values of, e.g., 3.33, 4.00 and 5.00 *seem* to be much more different from each other than the corresponding inverse values of 0.30, 0.25 and 0.20, even though the set of peak area data is the same in both cases and there is no fundamental problem involved here.

This second disadvantage can be avoided by introducing so-called enantiomer fractions (EFs) which are the counterpart of the chromatographic purity values briefly mentioned above. The definition is

$$\mathrm{EF} = \frac{\text{Peak area of enantiomer 1}}{\text{Peak areas of enantiomers 1} + 2} \times 100\%. \qquad (4)$$

Also in this case, the peak area corresponding with the limit of detection rather than zero should be used if the enantiomer is not found.

In principle, e.e. can also be used, with the sign of its value indicating which of the two enantiomers is dominant. However, often only absolute values are presented (dotted line in Fig. 1) which is of course a disadvantage (Bicchi et al., 1994). In addition, recording the percent difference of the two enantiomers is less straightforward than reporting the percentages themselves.

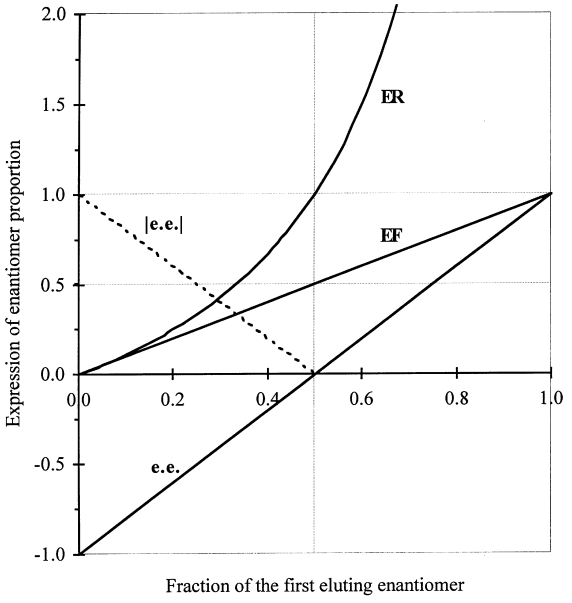

Fig. 1. Graphical representation of the various expressions used to indicate the enantiomer proportions plotted against the fraction of the first eluting enantiomer; the e.e. and EF values are usually expressed as percentages.

3. Examples

When calculating the mean ER value it is important to use the raw data instead of the calculated ER values, which is not always done correctly in the literature. On the other hand, the mean value can be calculated directly from the EF values and the raw data are not necessary, which obviously is an advantage. When, for example, a duplicate measurement is performed and the areas (in arbitrary units) of the enantiomers are 12.5 and 10.0 in the first run, and 10.0 and 12.5 in the second run, ERs of 1.25 and 0.80 result, and the mean value becomes 1.02 instead of 1.00. However, the EFs which are calculated to be 61% and 39%, give the correct mean value of 50%. Admittedly, the error inherent in the ER calculation can be prevented by first averaging the individual peak area measurements, but this is not always done. To quote an example taken from the literature (in which individual ER data are presented), Kallenborn et al. (1994) reported 10 repetitive measurements of 2-endo,3-exo, 5-endo,6-exo,8,8,10,10-octachlorobornane (B[12012]-(202) according to Wester et al. (1997)) and 2-endo,3-exo, 5-endo,6-exo,8,8,9,10,10-nonachlorobornane (B[12012]-(212)) in seal blubber, and calculated the mean ER values to be 1.024 and 1.059, respectively. The corresponding mean EFs – which can be calculated using $\mathrm{EF} = 100 \times \mathrm{ER}/(1 + \mathrm{ER})$ – are 50.59% and 51.43%, respectively. In this case only small deviations are found when calculating these EFs back to correct mean ERs of 1.0238 vs. 1.0240 and 1.0585 vs. 1.0590. The deviations are rather small because of the relatively small standard deviations (SDs) of the experimental data in the quoted study, which were 0.023 and 0.033, respectively. Not unexpectedly, such deviations increase with increasing relative standard deviations (RSDs): in the earlier example with the 1.25 and 0.80 duplicate measurement, the RSD is 31%. This causes the difference of 0.02 between the directly calculated 1.02 and the re-calculated (1.00; via EF) value.

It is interesting to add that the calculation of the SD itself can also lead to (seriously) incorrect values. In the quoted paper, the SDs of the measurements correspond with RSDs of 2.3% for B[12012]-(202) and 3.1% for B[12012]-(212). The correct RSDs derived from EF-based calculations are, however, only half as large, i.e., 1.1 and 1.5%, respectively.

The problem that mutual differences between a number of experimental data *suggest* much larger differences in enantiomer excesses than are actually present

Table 1
ER values of *cis*-heptachlorepoxide and α-HCH in eight roe-deer livers (*Capreolus capreolus*) caught in Schleswig-Holstein, Germany, 1992–1993 (Pfaffenberger et al., 1994) and calculated EF values

Sample no.	*cis*-Heptachlorepoxide		α-HCH	
	ER (+)/(−)	EF	ER (+)/(−)	EF
1	1	50.0	0.15	13.0
2	2	66.7	0.06	5.7
3	6	85.7	0.06	5.7
4	2	66.7	0.03	2.9
5	7	87.5	0.04	3.8
6	9	90.0	0.07	6.5
7	5	83.3	0.40	28.6
8	5	83.3	0.35	25.9
Mean	3	76.6	0.13	11.5

for ER values larger than unity, compared with differences between mutual ER values smaller than unity, can be illustrated with ER values of *cis*-heptachlorepoxide and α-HCH taken from the literature Pfaffenberger et al. (1994) and presented in Table 1. The ER values for *cis*-heptachlorepoxide cover a very wide range of 1–9, but proper calculation via EF shows that all values are less than 2-fold different, viz. from 50% to 90%, a mean value of 77% with a modest RSD of 18%. For α-HCH, on the other hand, the ER values, which also are mutually about one order of magnitude apart (0.03–0.40), do indicate real differences in measured enantiomer excess, as shown by the EFs of between 2.9% and 28.6% and the RSD of 88% (the mean EF, 11.5%). The striking differences, and the different appreciation, are caused by the non-linear scale of ER values. As can be seen in Fig. 1, a small variation in the relative amounts in which the enantiomers are present gives a small change in the ER value when the ERs are smaller than 1, and a large change in the ER when the ERs are larger than 1. On the other hand, the linearity of the EF scale causes the changes to be the same both above and below the racemic value.

4. Summary

Using enantiomer fractions, EFs, rather than enantiomer ratios, ERs, has the advantage that plots of EF vs. the fraction of an enantiomer are linear, that there are no undefined values anymore, that correct mean and standard deviation values are obtained and that equal excesses of enantiomer 1 or enantiomer 2 will immediately be recognised because the deviation from the racemic value of 50% will be the same.

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