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CHALLENGES IN ENANTIOSEPARATION AND DATA MANAGEMET OF CHIRAL POPS IN ENVIRONMENTAL STUDIES

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Introduction

Chiral environmental chemistry is a growing research area since chirality has been used extensively to quantify and characterize environmental biochemical processes¹. Enantioselective degradation of chiral pollutants plays an important role in environmental monitoring studies considering the re-emission of pollutants from secondary sources (e.g. soil, sediment, water, ice and plants). For that reason, the usage of chiral pollutants as tracers of environmental pollution is discussed inclusively by scientific researchers. In these studies, it is predicted that the fate and transport mechanisms of pollutants should be evaluated by chiral chemicals, i.e. the property of being racemic (newly used or protected from microbial degradation) or non-racemic (formerly used or exposed to microbial degradation)^{2,3}. Chiral separation is applicable for understanding the fate of pollutants; on the other hand, it is one of the most challenging tasks for any analytical technique especially in the field of biomedical, pharmaceutical, and environmental where pure enantiomeric forms are widely used⁴. Both of the enantiomers are treated as separate compounds, but their resolution is very difficult due to the same physical and chemical properties⁴. Thus, the measurement of individual enantiomers is a demanding area of significant interest⁵. Various enantioseparation methods have been used for different chemicals such as capillary electrophoresis, physical separation, and chromatographic separation, but chromatographic separation has been the most widely used among them⁶. In spite of its drawbacks, gas chromatography (GC) is the most widely used method in determination of chiral POPs since most of the environmental pollutants and their derivatives are volatile at the working temperature of GC^{4,7}. Some approaches such as, multidimensional GC, mass spectrometry detection, and liquid chromatography enrichment are recommended to overcome its drawbacks^{4,7}. The first step in the enantiomeric separation is development of analytical methods capable of separating enantiomers and providing reliable enantiomeric fraction (EF) values⁴. EF is the preferred metric for quantifying and evaluation whether being racemic or not⁵. The accuracy in determining the exact peak areas for enantiomers is very important since slight errors effect the calculation of EF⁵. Coelution of compounds in complex environmental mixtures may result in wrong determination⁸. For routine analysis, complete separation of enantiomers is impractical due to the nature of samples⁵. Besides, chiral columns are highly sensitive to moisture and dirty matrices⁸. For peak integration, the most commonly used peak integration method is "valley drop method" while a less commonly used method, namely "deconvolution method" is more accurate integration technique where incomplete separation and/or asymmetry is unavoidable⁵. Moreover, resolution and precision of peaks are worsened with decreasing signal to noise ratio, as expected⁵. All the things considered and results of literature survey reveals challenges in chiral analysis such as coelution of peaks in complex mixtures, sensitivity of chiral column to moisture and matrix components, low resolution, incomplete separation of enantiomer peaks, temperature dependency of the resolution of some chemicals, derivatization of the pollutant is time consuming, selection of peak integration method, low S/N ratio, asymmetry of peaks at racemic standards.

The aims of this study are to evaluate the challenges in enantioseparation, analytical procedure and data handling during analysis of chiral organochlorine pesticides based on literature and to bring a new perspective with the results of an air monitoring study. This is among the first studies on chiral analysis conducted in an environmental medium in Turkey.

Materials and methods

Sampling and analysis

The details of sampling, sample extraction, clean-up and instrumental analyses were presented elsewhere⁹. To summarize, a total of 47 samples were collected from 12 sampling sites using passive air

samplers (PAS) consisting of polyurethane foam (PUF) disks (14 cm diameter; 1.35 cm thick; surface area 365 cm²; volume 207 cm³; density 0.0213 g.cm⁻³) in an agricultural region in Kumluca, Antalya during one year period. Samplers were placed as far as possible from any potential contamination sources. Deployment was conducted in four periods for approximately 90 days from Mar 17, 2014 to Mar 14, 2015. After sampling, all samples were Soxhlet extracted using 400 ml of 1:1 acetone/hexane for 18 hours. Extracts were concentrated on rotary evaporator and purified under nitrogen stream until a volume of 1 ml achieved. Following alumina clean up (1 g of 6% deactivated) extracts were concentrated to 1 ml and exchanged into isoctane. 24 ng of PCB 121 was used as internal standard. Quantitative analysis of samples were carried out with a Shimadzu QP2010 Ultra gas chromatography-mass spectrometry (GC-MS) equipped with electron impact detector using a Restek Rxi 5Sil MS (60 m*0.25 mm*0.25 μm) capillary column. 5 chiral OCPs (o,p'-DDD, o,p'-DDT cis-chlordane (CC), trans-chlordane (TC) and α-HCH) out of 28 OCPs screened were subjected to enantiomeric separation. All samples with chiral OCPs concentrations above detection limit were analysed. Enantiomeric composition was determined with GC-MS using a BGB-172 chiral column (15 m*0.25 mm*0.25 μm). The GC oven temperature program for enantiomer separation was as follows: start at 90°C and increase 20°C.min⁻¹ to 170°C, 1°C.min⁻¹ to 180°C, 20°C.min⁻¹ to 230°C and lastly 1°C.min⁻¹ to 240°C. The injection port and interface were kept at 250°C, and ion source was at 230°C. Selected ion monitoring was used with the following ions: o,p'-DDD and o,p'-DDT (235, 237); CC and TC (373, 375); α-HCH (219, 181). The peaks were manually integrated with LabSolutions software using valley drop method.

Quality Assurance/Quality Control

¹³C₁₂-labelled PCB congeners (28, 52, 101, 153, 138, 180) (10 ng each) and d₈- p,p'-DDT (10 ng) were used as recovery surrogate to control the performance of the method during extraction and clean-up. Average recovery of seven surrogate compounds was 88.3±8.45% (ranged between 72.1% and 102%) for all samples. Recovery factor were not applied to any of the data. Analytical methods were validated using a certified reference material (CRM) including OCP (Fluka, CRM818) compounds. Results yielded an average recovery of 115±44.5% for OCP compounds present in CRM. The risk of contamination due to sampling and analysis was investigated by field (n=4) and laboratory (n=11) blanks.

Quality control issues with enantiomeric analysis were determination of method reproducibility and elimination of interferences. The calibration standards were assumed to be racemic mixtures that all enantiomers contributed equal parts to the total concentrations⁶. Therefore, the method reproducibility was determined by repetitive injection of racemic standards for detection of EF (EF = the ratio of peak areas; E1/(E1+E2)) (Figure 1). Unlike from the literature, standards having different concentrations which selected based on the detected OCPs levels in samples were used instead of a certain concentration. Freedom of peaks from interferences was ensured by requiring that the ratios of target/qualifier ions (Ion ratio, IR) for each enantiomer peak were within the 95% of calibration standards for a satisfactory analysis; otherwise, the result was rejected¹⁰. The effect of standard concentration on EF and IR were evaluated statistically by simple linear regression analysis and a p-value of less than 0.05 was considered to be statistically significant. The EF values were not linearly regressed against the concentration except α-HCH (R²= 0.732; p<0.05). On the other hand, IR values were linearly regressed against the concentration except CC (R²= 0.247; p>0.05).

Results and discussion

Of the analysed 5 chiral OCPs only o,p'-DDT and α-HCH were above detection limits at 12 and 4 samples, respectively (Figure 2). Two approaches were used in comparing EF values of samples with racemic standards. The first one is evaluation with considering the concentration of calibration standard and OCPs in samples. The second one is comparison with a constant EF value of a certain concentration (25 ng.ml⁻¹ was used since majority of samples are around this level). As depicted in Figure 3, concentration dependent evaluation does not cause a change in the results of evaluation for α-HCH. In both cases, all of the EF values are out of the racemic range indicating depletion of first enantiomer. On the other hand, with a few exceptions the EF values of o,p'-DDT are different than racemic range and the same for two different evaluation. However, one sample (S2-7) differs according to concentration based evaluation. It is racemic according to the evaluation based on a constant standard concentration, but depletion of first enantiomer is seen if the concentration of sample is taken into account for EF evaluation. It is worth to say that the results may differ according to the concentration based evaluation. Therefore, concentration

based evaluation is recommended for the interpretation of EF results in order to get more accurate results in the data management part of chiral analysis.

The relationship between EF values and air concentrations of chiral OCPs were evaluated (Figure 3). The EF values are not linearly regressed against the concentration in samples either for α -HCH and o,p'-DDT. Kurt-Karakus et al. (2005) reported similar results for global soil samples and suggested that EFs are not a good measure of overall degradation rates¹⁰. On the other hand, Shen et al. (2009) and Yuan et al. (2014) recorded a relationship between EFs and OCPs concentrations in soil and grass samples^{11,12}. Within this study, it can be concluded that EFs are not a good measure of overall degradation rates for α -HCH and o,p'-DDT in air samples.

In conclusion, concentration of racemic standard may affect the IR ratio and EF values for repetitive injections. Thus, using a concentration based calculation and evaluation is recommended for future studies rather than a constant concentration and/or using 0.5 for evaluation whether a pollutant is racemic or not. The details about the integration of enantiomer peaks such as integration method for each chemical should be specified in QA/QC. Overall EF results in air samples show depletion of enantiomers suggesting a former use except two samples. Racemic values gathered might be indication of fresh input or equal/similar depletion of enantiomers for these samples.

Acknowledgements

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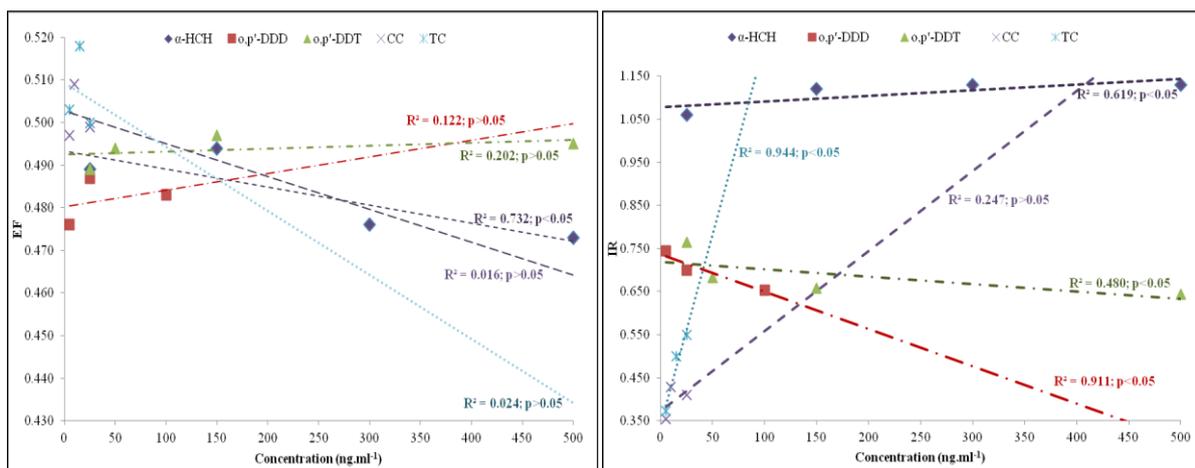


Figure 1. EF and IR values acquired by repetitive injection of calibration standards at different concentrations

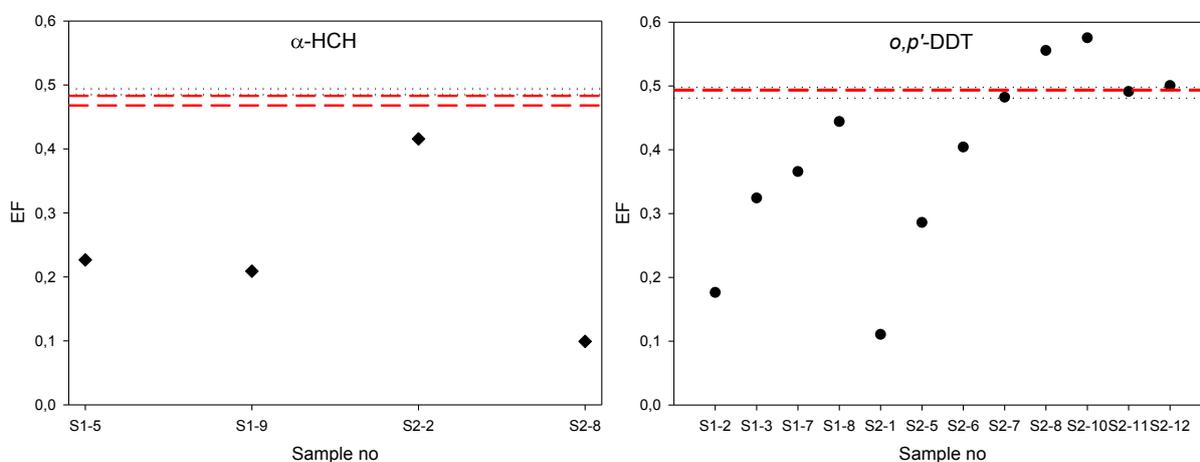


Figure 2. EF values of α -HCH and *o,p'*-DDT in samples and comparison with racemic values (black lines show considering a constant concentration and red lines show the racemic range considering concentration)

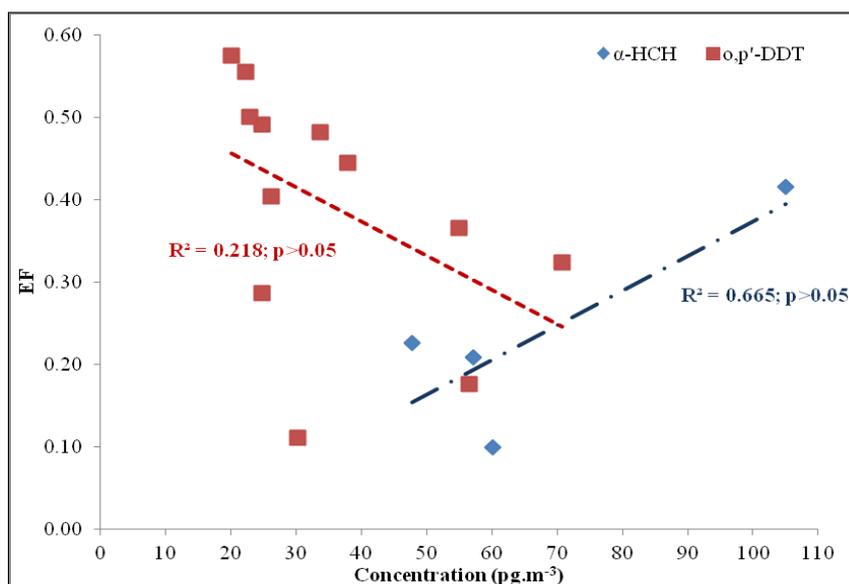


Figure 3. The relationship between EF and concentration