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Liquid chromatographic separation of the enantiomers of *trans*-chlordane, *cis*-chlordane, heptachlor, heptachlor epoxide and α-hexachlorocyclohexane with application to small-scale preparative separation

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Abstract

Analytical high-performance liquid chromatographic separations of the individual enantiomers of five polychlorinated compounds were obtained on polysaccharide stereoselective HPLC columns. The enantiomers of the pesticides *trans*-chlordane, *cis*-chlordane and heptachlor were separated on CHIRALCEL®OD using a hexane mobile phase. The enantiomers of the heptachlor metabolite, heptachlor epoxide, were separated on CHIRALPAK®AD using a methanol mobile phase. The enantiomers of α-hexachlorocyclohexane (α-HCH), were separated on CHIRALCEL®OJ using a hexane/2-propanol mobile phase. Similar chromatographic conditions using preparative columns were used to isolate approximately 250 mg of each of the individual enantiomers. The purified individual enantiomers have been submitted for testing of their endocrine disruptor (ED) activity.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Elution order; Organochlorine compounds; Pesticides; Endocrine disruptors; Polychlorinated hydrocarbons; Chlordane; Heptachlor; Heptachlor epoxide; α-Hexachlorocyclohexane

1. Introduction

While the majority of pesticides are achiral, a significant number are chiral and most of these compounds were sold or are sold as racemates [1,2]. Usually, there is a difference in the biological activity of the enantiomers [2,3] of these compounds because of stereoselective interaction with enzymes, cellular receptors or other chiral biological molecules. This selective interaction often results in differences in rates of metabolism or other biological transformations [4–8]; differences in bio-transport and bio-accumulation [8–10]; and differences in toxicity [11–13] of the two enantiomers.

Among other environmental concerns about polychlorinated xenobiotics is their effect on endocrine systems [2,14–20]. Compounds that can interfere with or disrupt the interaction of hormones are sometimes referred to as endocrine disruptors (ED) or endocrine moderators. While racemic *o,p′*-DDT has long been known to be estrogenically active [17], only a few chiral pesticides such as *o,p′*-DDT [18–20] and other chiral analogs of DDT [19] have been tested for enantioselective endocrine disruptor activity (chromatographic methods similar to those reported here were developed for the preparative and analytical separations of the enantiomers of some chiral analogs of DDT, *o,p′*-DDT, *o,p′*-DDD, *o,p′*-methoxychlor and *o,p′*-dicofol [21,22], for ED testing. The (−) enantiomer of *o,p′*-DDT was confirmed to be a much stronger estrogen mimic than the (+) enantiomer [19,20] while neither *o,p′*-DDD enantiomer showed strong ED activity [19]).

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To investigate the difference in biological activity of individual enantiomers it is necessary to obtain them in high purity by either isolating the enantiomers from the racemate or by stereoselective synthesis. In this paper, we describe enantioselective liquid chromatographic conditions that have been developed on polysaccharide chiral stationary phases (CSPs). These conditions are suitable for both analytical and preparative separations of the enantiomers of the pesticides *trans*-chlordane, *cis*-chlordane and heptachlor; heptachlor epoxide, which is a metabolite of heptachlor; and α-1,2,3,4,5,6-hexachlorocyclohexane (α-HCH) which is the only chiral 1,2,3,4,5,6-hexachlorocyclohexane and is a major component in technical HCH. One-hundred and ninety to 300 mg of each enantiomer of these compounds were isolated by preparative HPLC for testing their ED activity using a suite of assays designed to measure estrogen and androgen hormone activity [19].

Several recent reviews of enantioselective chromatographic separations of chiral pollutants including chlorinated chiral pesticides are available [23–26]. Gas chromatography has been used for enantioselective analytical separations [4–10,27–31] of the compounds discussed in this paper. Gas chromatography [10] and high-performance liquid chromatography [9,30] have been used for isolation of the individual enantiomers of these compounds (typically for use as retention time or order of elution standards in gas chromatography).

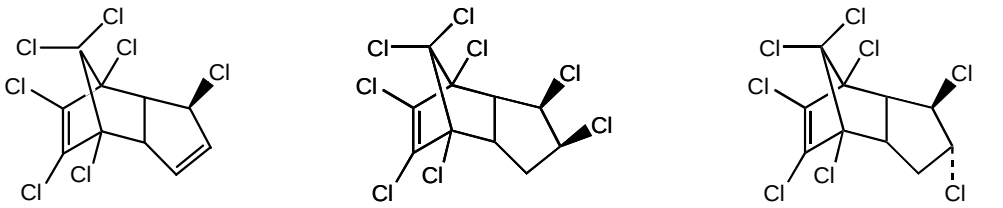
While gas chromatography is typically the technique of choice for analytical separations because of high column efficiency and high peak capacity, liquid chromatography is generally preferred for preparative separations since much larger loadings are possible [3]. In addition, in many cases, run times can be shorter in HPLC. In HPLC the mobile phase solvent plays a large role in chiral recognition [32,33]. This higher selectivity means that for the separation of a pair of enantiomers, shorter column length and/or less column efficiency is required in HPLC to achieve the same resolution as in GC.

In the work reported here, the enantiomers are identified by the sign of rotation of polarized light. The configurations of the (+) enantiomers of each of these pesticides are shown in Fig. 1. The absolute configurations of the enantiomers of the chlordane and heptachlor analogs were inferred by comparison of the CD spectra of their chiral precursors to the CD spectra of similarly bridged compounds of known absolute configuration [34,35]. The absolute configurations of the α-HCH enantiomers were determined by X-ray crystallography [9].

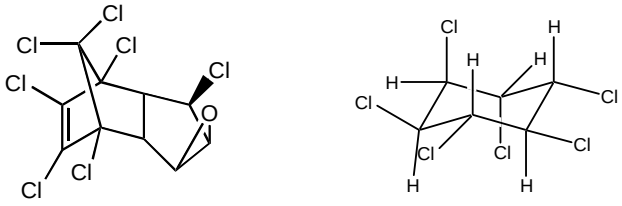
2. Experimental

2.1. Instrumentation

Two HPLC systems were used in method development. One was configured with a Shimadzu LC-10AT pump, Shimadzu SIL-10A variable injection volume autosampler (Shimadzu, Columbia, MD, USA) and Agilent photodiode array detector (Agilent Technologies Inc., Wilmington, DE, USA). The other was an Agilent 1100 HPLC system configured with a quaternary pump, mobile phase vacuum degasser, autosampler, temperature controlled column compartment, photodiode array detector and advanced laser polarimeter (ALP) chiroptical detector (PDR-Chiral, Lake Park, FL, USA). The wavelength of the laser in the in-line polarimeter is 675 nm. The sign of rotation of each of the enantiomers was obtained in mobile phase using the in-line polarimeter.



a. (+)-heptachlor b. (+)-*cis*-chlordane c. (+)-*trans*-chlordane



d. (+)-heptachlor epoxide e. (+)-α-HCH

Fig. 1. Structures of polychlorinated pesticides and related compounds.

The optical rotations in $CHCl_3$ using the sodium D line (589 nm) were obtained using a Model 341 Polarimeter from Perkin-Elmer (Boston, MA, USA). A 10 cm, 1 ml cell with a water jacket for temperature control was used. Temperature of the cell was maintained at 25 °C.

Preparative chromatography was performed using an AmeriChrom VERSAPrep I (Burtonsville, MD, USA), a Linear Instrument (Fremont, CA, USA) LINEAR^{TM} UVIS200 detector, automated injection and stand-alone fraction collector. Control of injection, fraction collection and processing of chromatographic data was performed using the AmeriChrom PSLC software (Version 3.21).

Gas chromatography/mass spectrometry (GC/MS) and gas chromatography/electron capture detector (GC/ECD) instruments were equipped with chiral capillary columns and used to test the identity and purity of the enantiomers obtained by preparative HPLC. *n*-Hexane (pesticide grade) solutions were prepared for each enantiomer at 0.1 mg/ml concentration; for both instruments, the injection volume was 1 μl. Helium (5-zeros grade) was used as the carrier gas.

For GC/MS, an Agilent 6890/5973 (Agilent Technologies Inc., Wilmington, DE, USA) was used in the electron impact mode at 70 eV to obtain total ion current chromatograms using a BGB 172 chiral column (BGB Analytik AG, Laufrainweg 139, 4469 Anwil, Switzerland) containing a chiral phase composed of 20% *tert*-butyldimethylsilylated-β-cyclodextrin. The column length was 30 m, i.d. was 0.25 mm, and film thickness was 0.25 μm. Column temperature was programmed from 150 to 220 °C at a rate of 1 °C/min for a total run time of 70 min. Injection was splitless at 250 °C and carrier gas flow was 1.0 ml/min. For each enantiomer solution, the total ion mass spectrum of any eluting peak was obtained and the major peak, assumed to be the enantiomer of interest, was matched with a library standard to assure correct identity. An attempt was made to identify any impurity peaks by interpretation of their mass spectra.

For GC/ECD, an Agilent 5890 (Agilent Technologies Inc., Wilmington, DE, USA) was used equipped with a Chirasil-Dex (Varian Chrompack, Raritan, NJ, USA) column coated with immobilized permethyl 2,3,6-tri-*O*-methyl β-cyclodextrin on a polysiloxane backbone as the stationary phase. Column length was 25 m, i.d. was 0.25 mm, and film thickness was 0.25 μm. Column temperature was programmed from 150 to 200 °C at 1 °C/min with a final hold of 25 min for a total run time of 75 min. Injection was splitless at 250 °C, carrier gas flow was 2 ml/min, and the detector temperature was 325 °C. The separated α-HCH enantiomers were also analyzed by GC/ECD using a cold on-column injection technique to demonstrate that impurities observed using an injection port temperature of 250 °C were due to thermal degradation in the injection port.

2.2. Solvents and mobile phase

HPLC grade hexanes (85% *n*-hexane) was obtained from Burdick & Jackson, Muskegon, MI, USA. 2-Propanol (IPA) was obtained from Pharmco Products, Brookfield, CT, USA. Denatured ethanol (EtOH) and methanol (MeOH) were obtained from JT Baker Phillipsburg, NJ. HPLC grade chloroform was obtained from Fisher Scientific Inc. (Atlanta, GA, USA). All solvents were used as received. All hexanes/IPA mobile phase solvent ratios are volume/volume (v/v). In method development, the mobile phase solvent compositions of 90/10, 95/5 and 97/3 hexanes/alcohol were premixed. The 98/2 and 99/1 hexanes/IPA mobile phases were obtained by having the instrument proportion 97/3 hexanes/IPA and hexanes to give the desired mobile ratio composition. For the preparative separations, the mobile phase solvents were premixed.

2.3. Pesticides and related substances

α-Hexachlorocyclohexane was obtained from ChemServices Inc. (West Chester, PA, USA). The other compounds were obtained from the EPA National Pesticide Standard Repository, US EPA, Environmental Sciences Center, 701 Mapes Road, Fort Meade, MD 20755-5350, USA. For screening studies the samples were prepared at 2–5 mg/ml in EtOH. For loading and stability studies the samples were prepared in mobile phase. Dilute solutions (1 μg/ml) of (+)-*trans*-chlordane and (−)-*trans*-chlorane in cyclohexane were obtained from Dr. Ehrenstorfer (EQ Laboratories Inc., Atlanta, GA, USA).

2.4. Chiral HPLC columns

The following HPLC columns (Chiral Technologies Inc., Exton, PA, USA) were used: CHIRALPAK®AD (amylose *tris*(3,5-dimethylphenyl carbamate)), CHIRALCEL®OD (cellulose *tris*(3,5-dimethylphenyl carbamate)) and CHIRALCEL®OJ (cellulose *tris*(4-methyl benzoate)). The analytical columns were 4.6 mm i.d. × 250 mm length, 10 μm particle size. Dimensions and the particle size of the CSP for the preparative columns are shown in Table 1.

3. Results and discussion

3.1. Analytical separations

As shown in Fig. 1, the compounds investigated in this paper all have similar structures (i.e., highly chlorinated, cyclic compounds). The enantiomers of the lipophilic pesticides, *trans*-chlordane, *cis*-chlordane and heptachlor are separated using an alkane mobile phase, the enantiomers of α-HCH are separated using a slightly more polar alkane/alcohol mobile phase, and the enantiomers of the more polar heptachlor epoxide are separated using an alcohol mobile phase. The

Table 1
Chromatographic conditions for preparative separations

Compound	Column	Column dimensions	Particle size (μm)	Mobile phase[a]	Flow rate (ml/min)
trans-Chlordane	CHIRALCEL®OD	2.1 cm × 25 cm	20	Hexanes	20
cis-Chlordane	CHIRALCEL®OD	2.1 cm × 25 cm	20	Hexanes	20
Heptachlor	CHIRALCEL®OD	10 cm × 50 cm	20	Hexanes	120
α-HCH	CHIRALCEL®OJ	2.0 cm × 25 cm	10	Hexanes/IPA 90/10 (v/v)	20
Heptachlor epoxide	CHIRALPAK®AD	2.0 cm × 25 cm	10	MeOH	10

[a] Separation performed at room temperature (~22 °C).

conditions for the enantiomeric separations of these five compounds are shown in Table 2. The chromatograms for these conditions are shown in Figs. 2–4.

Heptachlor, *trans*-chlordane and *cis*-chlordane were separated using the CHIRALCEL®OD column. The effect of decreasing IPA in the hexanes/IPA mobile phase on the separation of the enantiomers of *cis*-chlordane is shown in Fig. 2. As the IPA level is reduced from 5 to 3% (Fig. 2A) to 1% (Fig. 2B) in the mobile phase, a slight increase in resolution is observed. With a mobile phase of 100% hexanes (Fig. 2C), there is a large increase in retention and separation of the enantiomers along with a significant increase in peak width. While there is better separation for *cis*-chlordane using the 100% hexanes mobile phase, the peaks are sharper and the retention times are shorter using 99/1 hexanes/IPA. Sharper peaks are generally considered superior for an analytical separation since the peaks are easier to integrate and the limit of detection is lower. Greater separation is often preferable for a preparative separation since it permits greater sample loading, which generally increases production rates.

More interestingly, for *trans*-chlordane there is actually a reversal of elution order going from a mobile phase of hexanes/IPA 99/1 to hexanes. Fig. 3 shows separations of a *trans*-chlordane sample enriched in the (+) enantiomer. In Fig. 3A and B (hexanes/IPA mobile phase 97/3 and 99/1, respectively) the (+) enantiomer elutes first while in Fig. 3C (hexanes mobile phase) the (−) enantiomer elutes first. To demonstrate that the order of elution reversal was not an anomaly observed on a single CHIRALCEL®OD columns, the experiments were repeated on seven other CHIRALCEL®OD columns, including CHIRALCEL® 20 μm and CHIRALCEL®OD-H. The same effect was observed on all the columns.

The reasons for the dramatic change in chromatography going from 99/1 hexanes/IPA to 100% hexanes for *cis*-chlordane and the reversal of elution order for *trans*-chlordane were not investigated. However, it is well established that, as with other chiral stationary phases, the separation on the polysaccharide CSPs involves the interaction of the chiral stationary phase, the mobile phase solvent and the analyte [32,33,36]. While *cis*-chlordane and *trans*-chlordane do not have functional groups that can readily form hydrogen bonds with the alcohol in the mobile phase, the presence or absence of alcohol can affect solvation and subtle changes in the conformation of the CSP and thus how it can interact with the analyte.

The separation of the heptachlor epoxide enantiomers was obtained using CHIRALPAK®AD with 100% methanol as the mobile phase. A simple factorial experimental design [37] was used in an attempt to optimize the separation of this compound. Experiments were performed using (1) 3/1 MeOH/EtOH at both 15 and 35 °C, (2) 1/3 MeOH/EtOH at both 15 and 35 °C, and (3) 1/1 MeOH/EtOH at 25 °C (used as the "center point" of the experimental design). Neither the alcohol ratio nor the temperature had dramatic effect on the separation of heptachlor epoxide, therefore 100% MeOH at 25 °C was chosen for convenience. A chromatogram obtained using those conditions is shown in Fig. 4A.

The separation of heptachlor enantiomers, also using hexanes as the mobile phase on CHIRALCEL®OD, is shown in Fig. 4B. The separation of α-HCH enantiomers on CHIRALCEL®OJ, using 90/10 hexanes/IPA as the mobile phase, is shown in Fig. 4C.

Table 2
Chromatographic conditions for analytical separations

Compound	Column[a]	Mobile phase[b]	k_1	k_2	α	Elution order[c]
trans-Chlordane	CHIRALCEL®OD	Hexanes/IPA 99/1 (v/v)	0.83	0.93	1.1	+, −
trans-Chlordane	CHIRALCEL®OD	Hexanes	2.4	2.9	1.2	−, +
cis-Chlordane	CHIRALCEL®OD	Hexanes/IPA 99/1 (v/v)	0.87	1.0	1.2	+, −
cis-Chlordane	CHIRALCEL®OD	Hexanes	1.7	3.2	1.9	+, −
Heptachlor	CHIRALCEL®OD	Hexanes	0.80	0.97	1.2	+, −
α-HCH	CHIRALCEL®OJ	Hexanes/IPA 90/10 (v/v)	1.0	1.4	1.4	+, −
Heptachlor epoxide	CHIRALPAK®AD	MeOH	0.37	0.64	1.7	+, −

[a] Column dimensions 4.6 mm × 250 mm, 10 μm particle size.
[b] Heptachlor separation performed at room temperature (~22 °C), all other separations performed at 25 °C. Flow rate 1 ml/min.
[c] Based upon sign of rotation in mobile phase and in $CHCl_3$.

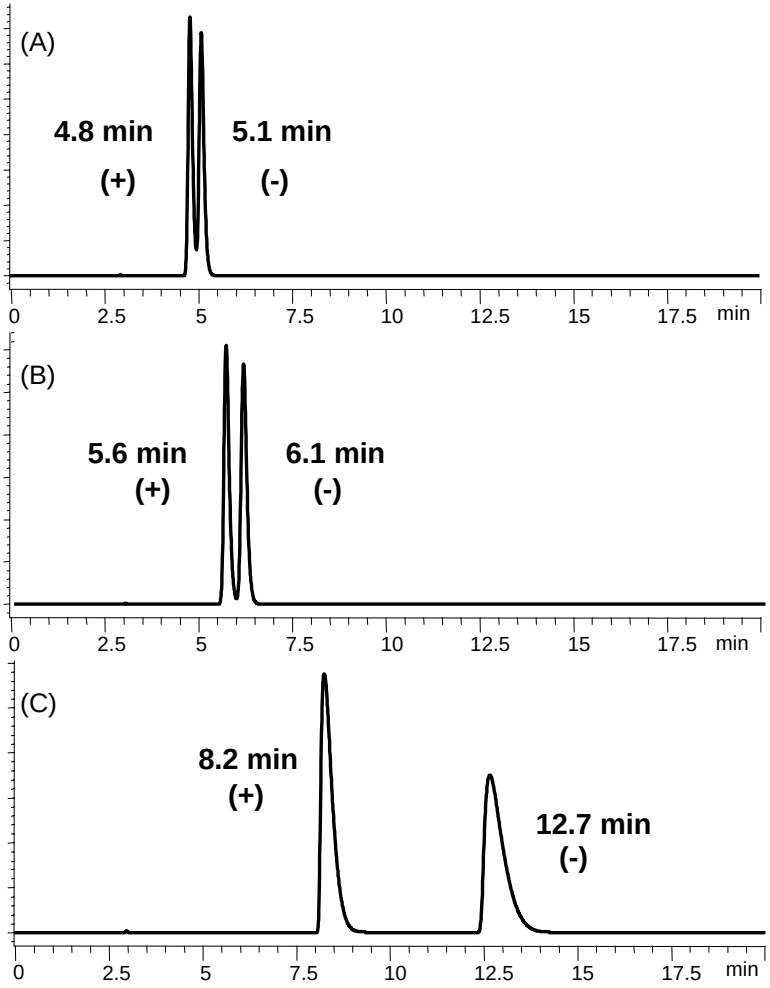


Fig. 2. Effect of IPA level in hexanes/IPA mobile phase on the separation of the enantiomers of *cis*-chlordane on CHIRALCEL®OD using hexanes/IPA mobile at flow rate of 1.0 ml/min, column temperature of 25 °C and with UV detection at 235 nm. Mobile phase: (A) hexanes/IPA 97/3 (v/v), (B) hexanes/IPA 99/1 (v/v), (C) 100% hexanes. The (+) enantiomer elutes first.

As shown in Figs. 3 and 4, there are impurities present in the *trans*-chlordane, heptachlor and α-HCH samples as received. These impurities were removed as part of the preparative separation. The impurities in *trans*-chlordane were shown by spiking, UV-spectrum and relative magnitude of optical rotation to UV response to be heptachlor.

3.2. Preparative separations

Chromatographic conditions for the preparative separations are shown in Table 1. The conditions were adequate to isolate at least 250 mg of each enantiomer. Except for dimensions of the columns, the chromatographic conditions for the preparative separations are similar to the analytical conditions shown in Table 2. 10 μm particle size CSP was used for the preparative separations of heptachlor epoxide on CHIRALPAK®AD and of α-HCH on CHIRALCEL®OJ. 20 μm particle size CSP was used for the other preparative separations. *trans*-Chlordane and *cis*-chlordane were separated on a CHIRALCEL®OD preparative column using 100% hexanes. For the other compounds, the preparative mobile phase compositions were the same as the analytical mobile phase compositions. As on the analytical column (i.e., 10 μm CSP), the (−) *trans*-chlordane enantiomer eluted first on the preparative scale column (i.e., 20 μm CSP) using a hexanes mobile phase.

3.3. Confirmation of identity and purity

The collected enantiomers in this study were determined to be greater than 98% pure by HPLC/UV analysis. However, since HPLC/UV can only detect impurities with a UV chromophore, GC/MS (using a BGB 172 chiral column) and GC/ECD (using a Chirasil-Dex column) were used as orthogonal techniques. The isolated enantiomers were analyzed by GC/MS and GC/ECD to: (1) detect and characterize impurities not detected by HPLC/UV and (2) confirm the identity of each enantiomer by comparing its mass spectrum to spectral libraries and archived spectra.

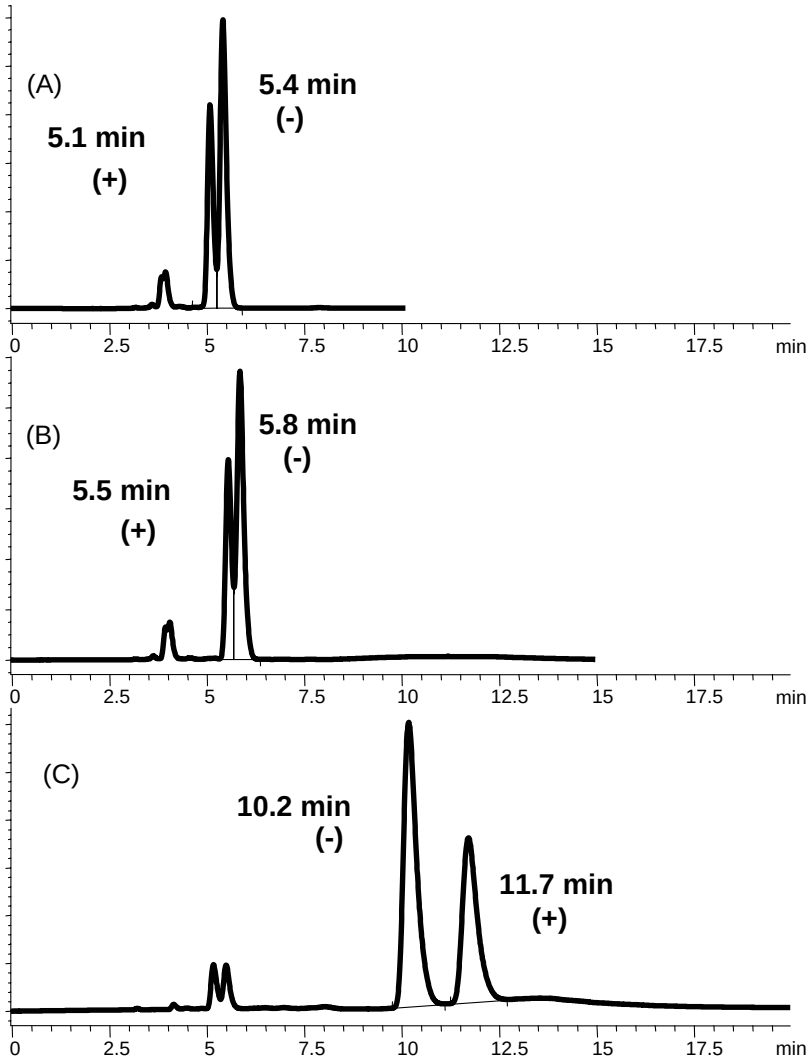


Fig. 3. Effect of IPA level in hexanes/IPA mobile phase on the separation and order of elution of the enantiomers of *trans*-chlordane on CHIRALCEL®OD using hexanes/IPA mobile at flow rate of 1.0 ml/min, column temperature of 25 °C and with UV detection at 235 nm. Mobile phase: (A) hexanes/IPA 97/3 (v/v), (B) hexanes/IPA 99/1 (v/v), (C) 100% hexanes. The (+) enantiomer elutes first in (A) and (B); the (−) enantiomer elutes first in (C).

The spectrum of each enantiomer clearly matched its library spectrum with a quality index of 98 or 99, except for the two α-HCH enantiomers, which matched at 93. Visual comparison of the mass spectra indicated almost perfect matches for all enantiomers. The only measurable impurity observed by MS was ∼2.9% of (+)-*trans*-chlordane, as identified by its spectrum and retention time, in the (−)-*trans*-chlordane enantiomer solution. The presence of (+)-*trans*-chlordane was also detected in the (−)-*trans*-chlordane sample by GC/ECD.

Analysis of the individual enantiomers of α-HCH using the BGB 172 column with MS total ion scan showed a spurious peak at 17.69 min, 2.1% of total scan area for the (+) enantiomer; and a spurious peak at 17.40 min, 2.2% of total scan area for the (−) enantiomer. Similar results were obtained using the Chirasil-Dex column with EC detection. These peaks evidently came from rapid thermal degradation of α-HCH in the hot GC injection port since the peaks were not observed when each α-HCH enantiomer was analyzed by GC/ECD using cold on-column injection.

The spectra corresponding to both degradation peaks gave a fair match with the library spectrum of δ-pentachlorocyclohexene, which is a dehydrochlorination product of α-HCH. It was assumed that the mass spectra of all the pentachlorocyclohexenes are similar, so it is not known whether the observed degradation product is the delta isomer or another isomer, such as is much more likely, β-pentachlorocyclohexene [3]. Cursory examination of the literature did not reveal reports of small amounts of these compounds being formed during the analysis of α-HCH.

Sign of rotation was obtained in mobile phase using the in-line PDR-Chiral ALP detector at 675 nm. The specific rotations (at 589 nm) have previously been reported in $CHCl_3$ for heptachlor [35], *cis*- and *trans*-chlordane [34] and heptachlor epoxide [34] and in acetone [38] and hexane [9] for α-HCH. To confirm that the solvent [39] and wavelength did

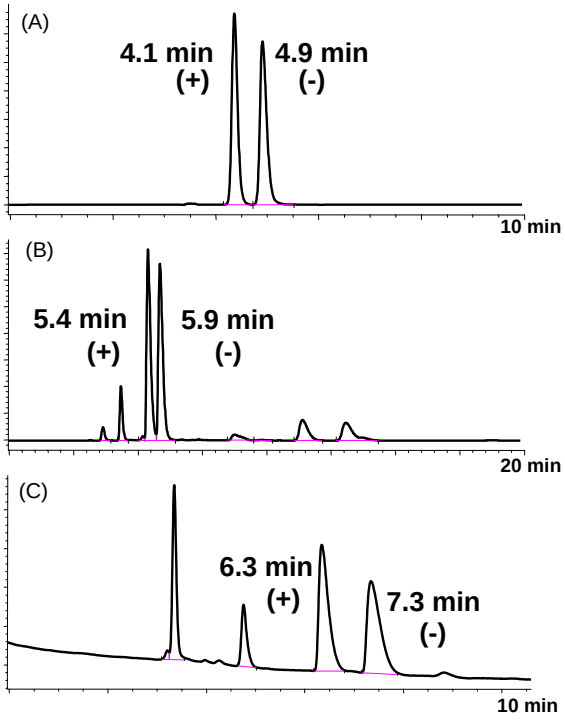


Fig. 4. (A) Separation of the enantiomers of heptachlor epoxide on CHIRALPAK®AD using MeOH mobile phase with UV detection at 235 nm. (B) Separation of the enantiomers of heptachlor on CHIRALCEL®OD using hexanes mobile phase with UV detection at 215 nm. (C) Separation of the enantiomers of α-HCH on CHIRALCEL®OJ using hexanes/IPA 90/10 (v/v) mobile phase with UV detection at 210 nm. All three chromatograms were obtained at a flow rate of 1.0 ml/min and a column temperature of 25°C for (A) and (C) and room temperature for (B). In each chromatogram the (+) enantiomer elutes first.

not affect the sign of rotation, specific rotation was obtained for each of the individual enantiomers in $CHCl_3$ and in mobile phase at 589 nm using the Perkin-Elmer 341 Polarimeter. The following results obtained in $CHCl_3$ are consistent with the reported results: (+)-*trans*-chlordane, $[\alpha]_D^{25}$ +3.0°; (−)-*trans*-chlordane, $[\alpha]_D^{25}$ −7.3°; (+)-*cis*-chlordane, $[\alpha]_D^{25}$ +60.0°; (−)-*cis*-chlordane, $[\alpha]_D^{25}$ −54.3°; (+)-heptachlor, $[\alpha]_D^{25}$ +205°; (−)-heptachlor, $[\alpha]_D^{25}$ −193°; (+)-heptachlor epoxide, $[\alpha]_D^{25}$ +93.5°; and (−)-heptachlor epoxide, $[\alpha]_D^{25}$ −96.4°. The sign of rotation and relative magnitude of rotation are consistent between $CHCl_3$ at 589 nm and mobile phase at 589 and 675 nm. Likewise, for α-HCH, the results obtained in $CHCl_3$ ((+)-α-HCH, $[\alpha]_D^{25}$ +120°; and (−)-α-HCH, $[\alpha]_D^{25}$ −129°) and hexanes/IPA 90/10 ((+)-α-HCH, $[\alpha]_D^{25}$ +113° and (−)-α-HCH, $[\alpha]_D^{25}$ −120°) are consistent with the results obtained in mobile phase at 675 nm and reported in acetone and hexane at 589 nm. The absolute reliability of the specific rotations reported here is limited because of the small weights (2–17 mg) and small volumes (1–1.5 ml) used.

The low magnitude of the specific rotation of *trans*-chlordane raised some concern that sign of rotation might not be a reliable way to distinguish the enantiomers. To confirm the material we labeled as (+) *trans*-chlordane and (−)-*trans*-chlordane are, respectively, the same enantiomers that are commercially available, we obtained authentic standards of the individual enantiomers from Dr. Ehrenstorfer. By gas chromatography (on both the BGB column with MS detection and the Chirasil-Dex column with EC detection) the material we considered the (+) enantiomer had the same retention as the (+) enantiomer from Dr. Ehrenstorfer and the material we considered as the (−) enantiomer had the same retention time as the (−) enantiomer from Dr. Ehrenstorfer. The standards were provided as 1 μg/ml solutions which was too dilute for reliable HPLC/UV analysis.

4. Summary

Separations adequate for analytical or for small-scale preparative chiral HPLC methods were obtained for the polychlorinated pesticides, *trans*-chlordane, *cis*-chlordane and heptachlor; for heptachlor epoxide (a heptachlor metabolite); and for α-HCH (a major component and the only chiral HCH in technical HCH). For the analytical separations, the analysis time is less than 15 min and in most cases less than 10 min. Better than baseline separation was obtained for the enantiomers of *trans*-chlordane, *cis*-chlordane, heptachlor epoxide and α-HCH. Near baseline separation was obtained for the enantiomers of heptachlor. The identity of the separated enantiomers was confirmed by GC/MS and GC/ECD. The sign of rotation for each of these compounds is the same in $CHCl_3$ and in mobile phase. Approximately 250 mg of each enantiomer was isolated for determination of endocrine disruptor activity; the individual enantiomers of these polychlorinated compounds are being tested for endocrine disruptor activity.

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