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High-performance liquid chromatographic separation of the enantiomers of organophosphorus pesticides on polysaccharide chiral stationary phases

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Abstract

High-performance liquid chromatographic separation of the individual enantiomers of 12 organophosphorus pesticides (OPs) was obtained on polysaccharide enantioselective HPLC columns using alkane–alcohol mobile phase. The OP pesticides were crotoxyphos, dialifor, fonofos, fenamiphos, fensulfothion, isofenphos, malathion, methamidophos, profenofos, crufomate, prothiophos and trichloronate. The enantiomers of fenamiphos, fensulfothion, profenofos and crufomate were separated on CHIRALPAK®AD®; the enantiomers of fenamiphos were also separated on CHIRALPAK®AS®; the enantiomers of methamidophos, crufomate and trichloronate were separated on CHIRALCEL®OD®; the enantiomers of crotoxyphos, dialifor, fonofos, malathion, prothiophos and trichloronate were separated on CHIRALCEL®OJ®; and the enantiomers of isofenphos were separated on CHIRALCEL®OG®. Baseline or partial separation of the enantiomers of six of these OP pesticides was obtained on CHIRALCEL®OJ. In continued method development, the separation of the enantiomers of the 12 OPs was investigated more extensively on CHIRALCEL®OJ to determine whether the mobile phase composition, flow-rate and column temperature could be optimized to yield at least partial separation of the enantiomers. Chromatographic conditions were found that gave either baseline or near baseline separations of the enantiomers of the 12 OPs on the CHIRALCEL®OJ column.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Organophosphorus pesticides; Polysaccharides

1. Introduction

Organophosphorus pesticides (OPs) were introduced in the 1950s for use on fruit, vegetable and other crops. Some of these pesticides are sold as the racemate (i.e. an equimolar mixture of the pair of enantiomers). The stereogenic (chiral) center in OPs may be pentavalent phosphorus or carbon, sulfur, or other atoms as a substituent of phosphorus [1]. Enantioselective biological recognition or biodiscrimination of enantiomers is often observed in biological systems. For example, relatively non-toxic racemic malathion is biotransformed to racemic

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malaoxon that has anti-acetylcholinesterase (insecticidal) activity. The *R*-maloxon enantiomer has more than a 22-fold greater inhibitory potency than *S*-maloxon for bovine erythrocyte cholinesterase [2]. The nerve agent, soman, has two centers of chirality (and thus four stereoisomers). The two (−) diastereomers of soman are potent inhibitors of acetycholinesterase and α-chymotrypsin; the two (+) diastereomers are only slightly toxic [3]. Racemic acephate does not inhibit acetylcholinesterase directly but is converted in insects to racemic methamidophos. The *R*-(+) enantiomers of acephate and methamidophos had the same absolute stereochemistry and were approximately 6-fold more toxic to houseflies than the *S*-(−) enantiomers [4].

The enantioselectivity observed in biological systems is determined by the stereochemistry of the biological systems (e.g. macromolecules such as cellular enzymes, cellular receptors or other macromolecules involved in membrane transport). The specific stereochemistry of the biological system can cause preferential interaction with one of the enantiomers over the other. Pharmacological and environmental fate studies are increasingly conducted on enantiomers because of the possible stereoselective efficacy and metabolism of each enantiomer [5]. Chromatographic separation of the enantiomers can be used to monitor enantiomerically selective degradation of racemates.

Separations of the enantiomers of several racemic OPs by gas chromatography [6,7], capillary electrophoresis [8], and high-performance liquid chromatography (HPLC) [4,9–12] have been reported. This manuscript describes the HPLC separation of the enantiomers of 12 OPs on polysaccharide chiral stationary phases (CSP). Initially a standard column-selection procedure that utilized the same chromatographic conditions on CHIRALPAK®AD, CHIRALPAK®AS, CHIRALCEL®OD and CHIRALCEL®OJ columns was used to determine CSP selectivity for the OP enantiomers. One of the OPs was not resolved in the standard column-selection study but was resolved on CHIRALCEL®OG®. Separation of the enantiomers of the 12 OPs was investigated further on CHIRALCEL®OJ column. This CSP that had shown at least partial separation for the enantiomers of the largest number of OPs. The experiments on this column demonstrated that the mobile phase composition, flow-rate and column temperature could be optimized to yield at least partial separation of all the OP enantiomers using only one of the enantioselective columns.

2. Experimental

2.1. Instrumentation and chromatographic conditions

2.1.1. System 1

The HPLC system used in the initial column selection studies was a Shimadzu LC-10AT pump, Shimadzu SIL-10A variable injection volume autosampler, and Shimadzu SPD-10A variable wavelength UV detector (Shimadzu, Columbia, MD USA). A polarimeter detector, supplied by PDR-Chiral (West Palm Beach, FL, USA) was used in series with the UV detector. The data system was a Rainin Dynamax® Dynamax® MacIntegratorJ I™R, Version 1.43 (Varian, Walnut Creek, CA USA). A 12-port column switching valve (VALCO, Houston, TX USA) was used to allow unattended column switching.

2.1.2. System 2

Two Hewlett-Packard 1100 HPLC systems configured with quaternary pump systems, mobile phase vacuum degasers, autosamplers, thermostatted column compartments and photodiode array detectors (Agilent Technologies, Inc., Wilmington, DE (USA) were used in the temperature and mobile phase composition studies. All parameters of the HPLC system were computer controlled using Version A.06.01 or Version A.08.01 software loaded on a LC^{3D} ChemStation. One of these instruments (System 2-A) was also equipped with a polarimeter detector supplied by PDR-Chiral; System 2-B was equipped only with a photodiode array detector.

2.2. Reagents and chiral organophosphorus pesticidess

Optima grade hexane, heptane, 2-propanol (IPA)

and denatured ethanol (EtOH) were obtained from Fisher Scientific (Fairlawn, NJ, USA) and used as received. The EtOH, as supplied, was denatured with 4.7% methanol (MeOH) and 4.8% IPA; actual ethanol content was 90.5%. The mobile phase used in initial screening conditions was heptane/EtOH 90/10 v/v. Eleven of the OPs were obtained from ChemService, West Chester, PA, USA. Dialifor was supplied by Dr Ehrenstorfer GmbH, Augsburg, Germany. Stock solutions of the OPs were prepared in hexane or heptane and dilutions of the stock solutions were made in hexane to obtain final concentrations of 0.1–1.0 mg/ml, unless otherwise specified.

2.3. Chiral stationary phase columns

The following HPLC columns (Chiral Technologies, Inc., Exton, PA USA) were used: CHIRALPAK®AD [amylose tris(3,5-dimethylphenyl carbamate)], CHIRALPAK®AS [amylose tris((*S*)-1-phenylethyl carbamate)], CHIRALCEL®OD [cellulose tris(3,5-dimethylphenyl carbamate)], CHIRALCEL®OG [cellulose tris(4-methylphenyl carbamate)] and CHIRALCEL®OJ [cellulose tris(4-methyl benzoate)]. The columns were 250×4.6 mm I.D. with the enantioselective phase coated onto a 10 μm silica-gel substrate.

2.4. Chiroptical detection

Absolute configuration of the enantiomers was not determined. The enantiomers are distinguished based upon sign of rotation of plane polarized light obtained by using an in-line polarimeter supplied by PDR-Chiral (West Palm Beach, FL, USA). The light source for this detector is a laser (675 nm); the cell path is 50 mm. Many factors can affect magnitude (and sometimes sign) of rotation. These factors include wavelength, solvent, temperature, pH and analyte concentration [1]. While the sign of rotation (i.e. (+) or (−) should not be used as a universal identification (such as *R* or *S*; or *D* or *L*) for each enantiomer, the sign of rotation was used to compare order of elution between columns since the chromatographic conditions were equivalent.

3. Results and discussion

3.1. Evaluation of enantioselective columns for separation of organophosphorus pesticides

The 12 chiral organophosphorus pesticides in Fig. 1 represent a range of pentavalent phosphorus functionally: phosphate (**1**), phosphorothioates (**5**, **9**), phosphorodithioates (**2**, **7**, **11**), phosphonothioate (**12**), phosphonodithioate (**3**), phophoroamidates (**4**, **10**), phosphoramidothioate (**6**), and phosphoroamidothioate (**8**). In eight of the OPs the stereogenic center (denoted by *) is pentavalent phosphorus; three have a chiral carbon in a substituent of phosphorus and in one OP the center of chirality is a sulfoxide group. The diversity expressed in their chiral centers would seem to require equally divergent enantioselective separation mechanisms in the enantioselective HPLC columns.

Several recent publications report the separation of the enantiomers of a large number of compounds on tribenzoate and triphenylisocyanate derivatized amylose- and cellulose-CSPs. The enantioselectivity of the derivatized polysaccharide-CSPs is attributed to the degree of steric fit of the enantiomers in the ''chiral cavity'' of the CSP. The enantiomer–CSP interactions and recognition mechanisms are discussed in terms of hydrogen bonding, dipole moments, π–π interactions, molecular level studies using nuclear magnetic resonance and mass spectroscopy, and computational chemistry [13–16]. Four polysaccharide-CSP HPLC columns reported to offer the highest degree of stereogenic recognition for the greatest number of compounds [15] are CHIRALPAK®AD, CHIRALPAK®AS, CHIRALCEL®OD and CHIRALCEL®OJ.

Separation of the enantiomers of the 12 phosphorus compounds was investigated using similar chromatographic conditions. A mobile phase of heptane/EtOH (90/10 v/v) at a flow-rate of 1 ml/min at room temperature was used for initial evaluation or screening of columns. In all experiments, other than the initial screening, the column temperature was controlled. The sign of optical rotation in the mobile phase (Table 1) for each enantiomer was determined using the polarimeter detector.

Using the screening conditions and the four col-

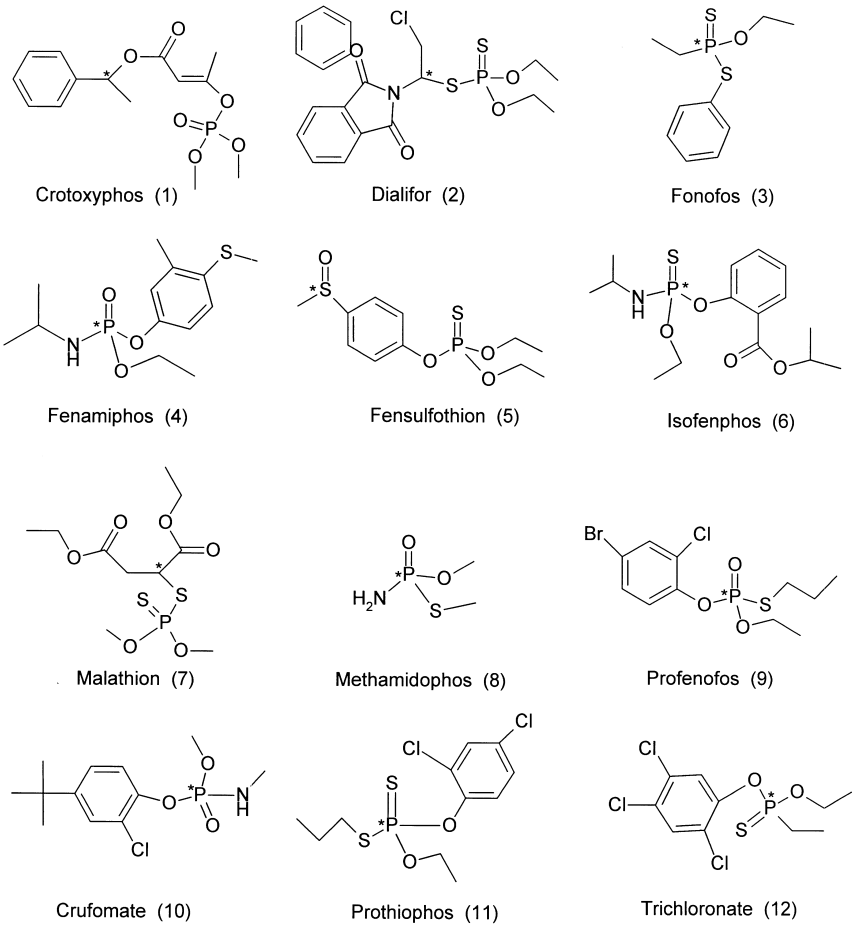

Fig. 1. Chiral organophosphorus pesticides; * denotes chiral center.

umns listed above, baseline resolution of the enantiomers was obtained for **1–5**, **7**, **8** and **10** (Table 1). With simple modification to the mobile phase and/or temperature, separation of the enantiomers of isofenphos (**6**), profenofos (**9**), and prothiophos (**11**) was increased but not to baseline separation. Isofenphos was, however, resolved on a 5th column, CHIRALCEL®OG, using 98/2 hexane/IPA as the mobile phase. A separation of isofenphos has been reported on CHIRALCEL®OC [10] and Sumipax OA-4000 [12]. Profenofos enantiomers (**9**) were almost baseline resolved on CHIRALPAK®AD using a 98/2 heptane/EtOH mobile phase at 15°C. Similarly, prothiophos enantiomers (**11**) were almost baseline resolved on CHIRALCEL®OJ using 98/2 heptane/EtOH mobile phase at 15°C. Baseline separation of the enantiomers of trichloronate (**12**) was obtained on both CHIRALCEL®OD and CHIRALCEL®OJ using 100% heptane and hexane, respectively, at 15°C. No optical rotation for either enantiomer was observed in heptane, hexane, or EtOH. The same order of elution of trichloronate was confirmed for both columns by collecting each eluted enantiomer, mixing in unequal ratios and reinjecting on each column.

Crufomate (**10**) was resolved using screening conditions on CHIRALPAK®AD and almost baseline resolved on CHIRALCEL®OD (Fig. 2). Note that on CHIRALPAK®AD the enantiomer showing (−) rotation in the heptane/EtOH mobile phase elutes first and on CHIRALCEL®OD the enantiomer showing (−) rotation in the heptane/EtOH mobile phase

Table 1
Separation of organophosphorus pesticides on polysaccharide chiral stationary phases, 25°C, 1 ml/min

Compound	°C	Column	Heptane/EtOH[a]	k_1	k_2	α	R_s	Rotation Pk1/Pk2
Crotoxyphos (**1**)	25.0	OJ	90/10	3.7	5.0	1.3	2.1	−/+
Dialifor (**2**)	rt[b]	OJ	90/10	2.3	2.8	1.2	1.3	+/−
Fonofos (**3**)	25.0	OJ	90/10	1.6	2.2	1.4	2.1	+/−
Fenamiphos (**4**)	rt	AS	90/10	1.6	2.8	1.8	1.7	−/+
	25.0	AD	95/5	2.5	3.0	1.2	2.0	+/−
Fensulfothion (**5**)	25.0	AD	90/10	3.2	4.4	1.4	2.1	−/+
Isofenphos (**6**)	25.0	OG	98/2[c]	0.8	1.0	1.3	1.1	+/−
Malathion (**7**)	rt	OJ	90/10	2.3	3.1	1.3	1.9	+/−
Methamidophos (**8**)	rt	OD	90/10	2.8	3.5	1.2	1.7	+/−
Profenofos (**9**)	15.0	AD	98/2	2.2	2.6	1.2	1.2	+/−
Crufomate (**10**)	rt	AD	90/10	1.5	1.9	1.3	2.8	−/+
	rt	OD	90/10	0.9	1.1	1.3	1.1	+/−
Prothiophos (**11**)	15.0	OJ	98/2	1.4	1.6	1.2	1.6	+/−
Trichloronate (**12**)	15.0	OD	100/0	1.8	2.1	1.1	1.3	ud[d]
	10.0	OJ	100/0	1.6	1.9	1.2	0.9	ud[d]

[a] Ethanol denatured with 4.7% methanol and 4.8% IPA.
[b] Room temperature (~22°C).
[c] Heptane/IPA.
[d] Rotation was undetermined, but order of elution determined by spiking was same on both CHIRACEL® OD and CHIRACEL® OJ.

elutes second. Chemically, CHIRALPAK®AD and CHIRALCEL®OD are similar. In both CSP's the free hydroxyls of the glucose polymer are derivitized to give 3,5-dimethylphenylcarbamate. The CHIRALPAK®AD polymer is amylose; the CHIRALCEL®OD polymer is cellulose. The other OPs that showed at least partial baseline separation on both CHIRALPAK®AD and CHIRALCEL®OD were compared to see if reversal in order of elution occurred. The results are summarized in Table 2. Crufomate (**10**) and fensulfothion (**5**) show a reversal of ''elution order'' between CHIRALPAK® AD and CHIRALCEL®OD; fenamiphos (**4**), methamidophos (**8**) and profenophos (**9**) do not show reversal of elution. In practice, reversal of order of elution between CHIRALPAK®AD and CHIRALCEL®OD is not uncommon [16]. Separation of methamidophos (**8**) has previously been reported on CHIRALCEL®OC [4]; however, the separation on CHIRALCEL®OD results in shorter analysis time.

Fenamiphos showed baseline separation on CHIRALPAK®AS using the screening conditions 90/10 heptane/EtOH; however better peak shape was obtained on CHIRALPAK®AD using 95/5 heptane/EtOH mobile phase. Note also as shown in Table 1 that elution order is reversed between CHIRALPAK®AS and CHIRALPAK®AD.

3.2. Effect of mobile phase and flow-rate on enantioselective separations on CHIRACEL®OJ

In the column-selection studies, baseline (**1–3** and **7**) and near baseline (**11** and **12**) separation of the OP enantiomers was obtained on CHIRALCEL®OJ (Table 1). Therefore, CHIRALCEL®OJ was selected for further method development to determine whether the mobile phase composition, flow-rate and column temperature could be optimized to yield at least partial separation of the enantiomers of all twelve OP pesticides. The additional method development on the separation of the enantiomers of the OPs was preformed on System 2-B, a different CHIRALCEL®OJ column and hexane rather than heptane was used with EtOH at 90/10 v/v at a flow-rate of 0.9 ml/min and 20°C. The separation factor (α) of **1–3**, **7**, **11** and **12** reported in Table 3, were comparable to results reported in Table 1 on the original CHIRALCEL®OJ column.

In general, for coated polysaccharide CSPs, a

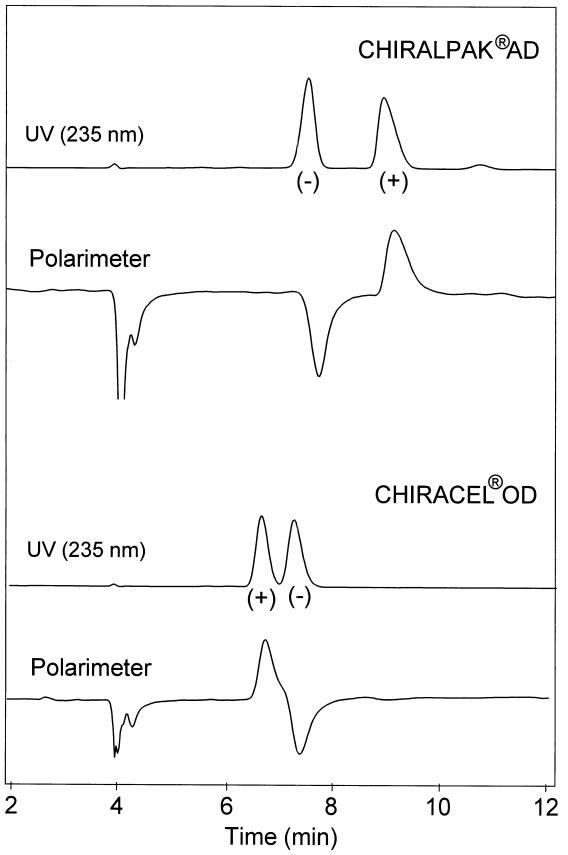

Fig. 2. The polarimeter chromatogram shows the reversal in order of elution of the enantiomers of crufomate on CHIRALPAK®AD and CHIRACEL®OD.

decrease in the amount of polar modifier in the mobile phase will have only a small effect on the separation while a linear increase in resolution is often observed; for some enantiomers a maximum resolution may be observed at a certain mobile phase polar modifier content [17]. The effect of mobile phase composition on the selectivity of the CHIRALCEL®OJ column for the enantiomers of **4–6** and **8–12**, was investigated by decreasing the EtOH content from 10 to 0% by volume in 1%-increments. Column temperature was maintained at 20°C and the column flow was maintained at 0.9 ml/min. When a partial separation was observed the EtOH was decreased in 0.5%-increments until the peak2/peak1 area ratio reached a constant value (maximum separation efficiency for the chromatographic conditions). Baseline resolution of the enantiomers of prothiophos (**11**) was obtained when the mobile phase EtOH content was decreased to 0.5%. However decreasing mobile phase EtOH to below 6.5% did not noticeably improve the enantioselectivity for methamidophos (**8**) while the retention time increased by a factor of three. At least partial resolution was obtained for **4–6**, **9**, **10** and **12**, as the EtOH content was decreased. However, trichloronate (**12**) eluted as an unresolved peak until the mobile phase composition reached 100% hexane where near baseline resolution was obtained at 5°C (Fig. 3). Similarly, for profenofos (**9**) the separation factor increased from 1.07 to 1.43 and resolution increased from 0.85 to 3.2 when the mobile phase was changed from hexane/EtOH 99/1 v/v to 99.5/0.5 v/v.

3.3. Effect of temperature on enantioselective separations on CHIRACEL®OJ

The effect of temperature on enantioselective separation must be investigated on a compound by compound basis [18–21]. For many chiral compounds a linear relationship is observed between the logarithm of the separation factor (α) of the enantiomers and the inverse of the temperature; separation of the enantiomers increases as the temperature of the separation is lowered [17,19]. The enanatioselective separation of **4–6**, **8–10** and **12** was investigated

Table 2
Comparison of order of elution on CHIRALPAK®AD and CHIRACEL®OD using heptane/EtOH

Name	CHIRALPAK®AD	CHIRACEL®OD	Reversal
Fenamiphos (**4**)	+/−	+/−	No
Fensulfothion (**5**)	−/+	+/−[a]	Yes
Methamidophos (**8**)	+/−[a]	+/−	No
Profenofos (**9**)	+/−	+/−	No
Crufomate (**10**)	−/+	+/−	Yes

[a] Incompletely resolved.

Table 3
Effect of mobile phase composition, temperature and flow-rate on enantioselectivity of CHIRACEL®OJ

Compound name	nm	Hexane/EtOH[a]	Flow (ml/min)	°C	k_1	k_2	α	R_s	Rotation Pk1/Pk2
Crotoxyphos (**1**)	211	90/10	0.90	20.0	3.49	5.12	1.47	5.64	−/+
Dialifor (**2**)	220	90/10	0.90	20.0	2.25	2.92	1.30	3.12	+/−
Fonofos (**3**)	202	90/10	0.90	20.0	1.77	2.64	1.49	5.52	+/−
Fenamiphos (**4**)	203	99.1/0.90	0.50	40.0	3.96	4.25	1.07	1.08	+/−
Fensulfothion (**5**)	201	96/4	0.80	40.0	6.64	7.12	1.07	1.21	−/+
Isofenphos (**6**)	201	99.4/0.60	0.30	10.0	1.74	1.93	1.11	1.11	+/−
Malathion (**7**)	210	90/10	0.90	20.0	2.37	3.19	1.34	4.11	+/−
Methamidophos (**8**)	200	93.5/6.50	0.80	5.0	5.71	6.47	1.13	1.56	+/−
Profenofos (**9**)	202	99.5/0.50	0.80	5.0	2.69	3.81	1.41	3.52	+/−
Crufomate (**10**)	203	99/1	0.30	10.0	3.35	3.58	1.07	0.90	−/+
Prothiophos (**11**)	202	99.5/0.50	0.70	20.0	1.30	1.50	1.33	4.00	+/−
Trichloronate (**12**)	205	100.0/0	0.80	5.0	1.33	1.65	1.24	1.40	ud[b]

[a] Ethanol denatured with 4.7% methanol and 4.8% IPA, v/v.
[b] Undetermined.

with stepwise lowering of the CHIRACEL®OJ column temperature from 20 to 15 to 5°C, while maintaining the optimum hexane/EtOH ratio determined in Section 3.1. The results were somewhat surprising; only methamidophos (**8**) showed a slight increase in enantioselectivity. The separation factor for (**8**) increased from 1.09 at 20°C to 1.13 at 5°C in the mobile phase 93.5/6.5 hexane/EtOH. The separation factor remained unchanged for **6**, **9**, and **10**, but enantioselectivity of the enantiomers was improved at the lower temperatures as shown by the improved symmetry of the second eluting enantiomer and the area ratio of peak2/peak1 approaching unity. Trichloronate (**12**) eluted as a single peak even at

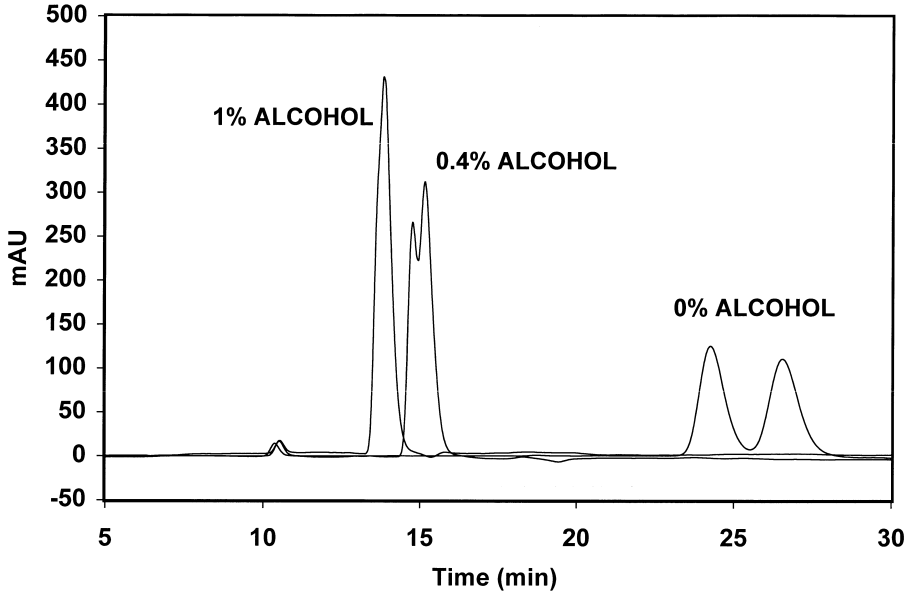

Fig. 3. The effect of polar modifier content on the separation of the enantiomers of trichloronate on CHIRACEL®OJ. Ethanol denatured with 4.7% methanol and 4.8% IPA.

5°C in 99/1 hexane/EtOH. When EtOH was decreased to 0.6%, partial separation was obtained but the peak2/peak1 ratio was 1.83. The enantiomer peak area ratio decreased to 1.04 in 100% hexane, at 5°C with a calculated separation factor $\alpha=1.24$ and $R_s=1.40$ at a flow-rate of 0.8 ml/min (Fig. 3).

A plate number $N=3704$ plates/column was calculated for the second eluting enantiomer of trichloronate (**12**). The plate number for the second eluting enantiomer of **12**, compared favorably with similar values of $N=4600$ and $N=4200$ for fensulfothion (**5**) and fenamiphos (**4**), respectively at 20°C and demonstrated that the column retained efficiency and selectivity at the lower temperature. The effect of mobile phase EtOH content on enantioselective separations was similarly demonstrated in the resolution of the enantiomers of profenphos (**9**) at 5°C. In 99/1 hexane/EtOH the peak2/peak1 area ratio was 1.25 with a separation factor of 1.07, and resolution of 0.85. When the EtOH content was decreased to 0.5% the peak2/peak1 ratio was unity, the separation factor improved to 1.43 and resolution to 3.2 (baseline).

When the column flow is decreased below the commonly used flow-rate of 1 ml/min, an increase in column efficiency is often observed [17]. The separation factor was 1.10 and the peak 2/peak1 area ratio was 1.37 for isofenphos (**6**) in 99.4/0.6 hexane/EtOH at 10°C and a column flow of 0.9 ml/min. When the column flow was decreased to 0.3 ml/min the separation factor remained constant but the peak2/peak1 area ration decreased to 1.03 (indicating increased peak symmetry for overlapping enantiomers). Both resolution and the peak2/peak1 area ratios of the enantiomers of eight of the 12 OPs was improved by dropping the flow-rate below 0.9 ml/min (Table 3). Similar improvement in peak symmetry and resolution was obtained for the enantiomers of fenamiphos (**4**) and fensulfothion (**5**) when column flow was decreased but, unfortunately, their retention times exceeded 40 min at 20°C.

The resolution of enantiomers of specific chemicals has been reported to increase when the temperature was incrementally increased from 20 to 65°C [20,21]. For example, the resolution of the enantiomers of *p*-hydroxy methyl sulphoxide on CHIRALCEL®OB and rolipram on CHIRALCEL® OD increased from single peaks at 20°C to baseline separation at 50 and 65°C, respectively. Interestingly, of 10 sulphoxide analogs studied [20] only the *p*-hydroxy derivative exhibited enhanced separation at elevated temperatures as did only two of nine derivatives of rolipram [21]. The effect of raising the column temperature in 10° increments was investigated on **4** and **5**. For fenamiphos (**4**) the separation factor remained constant at 1.07, resolution increased from 0.82 to 1.05 and 1.08, and the peak2/peak1 ratio decreased from 1.21 to 1.05, and 1.05 at column temperatures of 20, 30 and 40°C, respectively. The absolute retention time of peak 2 of fenamiphos decreased only 6 min from 36.7 min at 20°C to 30.7 min at 40°C. A slightly different response was observed for fensulfothion (**5**) as shown in Fig. 4. The separation factor remained constant at 1.07 as did the peak2/peak1 area ratio of 1.00 as the column temperature was increased from 20 to 60°C. Resolution increased from 1.0 at 20°C to 1.21 at 40°C and 1.28 at 60°C. However, the decrease in retention time of fensulfothion was much greater than for fenamiphos; the retention time for the second eluting enantiomer of fensulfothion decreased from 48.3 min at 20°C to 27.8 min at 40°C and 18.9 min at 60°C. The column efficiency calculated for peak 2 of fensulfothion (**5**) increased from $N=4624$ at 20°C to $N=6776$ at 60°C.

4. Summary

Baseline separation of the enantiomers of all 12 OPs was obtained. Standard conditions of mobile phase and temperature or slight modification of conditions gave baseline separation of the enantiomers of nine of the 12 OPs on the four columns typically used in screening studies. Complete resolution of the enantiomers of **4**, **5**, and **10** was obtained on CHIRALPAK®AD; complete resolution of the enantiomers of **4** was obtained on CHIRALPAK®AS; complete resolution of the enantiomers of **8**, **10** and **12** was obtained on CHIRALCEL®OD; and baseline resolution of the enantiomers of **1**, **2**, **3** and **7** was obtained on CHIRALCEL®OJ. Baseline resolution of the enantiomers of **6** was achieved on CHIRALCEL®OG; a column that is not used routinely in screening studies.

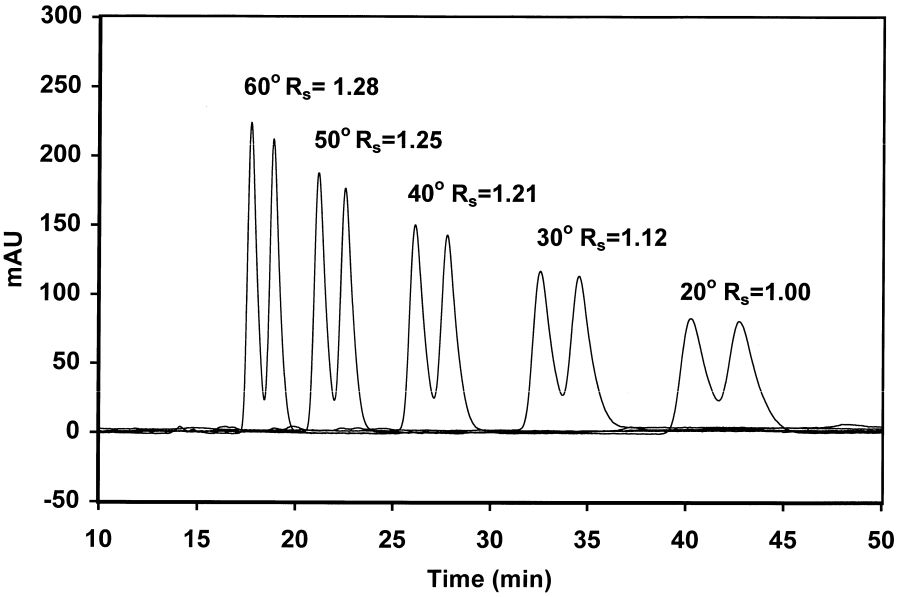

Fig. 4. The effect of temperature on the separation of the enantiomers of fensulfothion on CHIRACEL®OJ.

A more detailed study of the effects of temperature and solvent composition on enantioselective separation was performed on CHIRALCEL®OJ. Baseline resolution of the enantiomers of two additional OPs (**9** and **11**) was achieved by decreasing the mobile phase polar modifier to 0.5%. Baseline resolution of the enantiomers of a seventh OP (**12**) was achieved on CHIRALCEL®OJ with 100% alkane mobile phase. The resolution of the enantiomers of the remaining five OPs on CHIRACEL®OJ was improved relative to the screening conditions by optimization of both the mobile phase composition and column temperature. The resolution of the enantiomers of methamidophos (**8**) was improved to near baseline, while the smallest resolution (R_s = 0.90) was observed for the enantiomers of crufomate (**10**). A column temperature of 40°C shortened retention times and improved the resolution of the enantiomers of fenamiphos (**4**) and fensulfothion (**5**). The increase in column efficiency with increased column temperature was demonstrated by the increased resolution of fensulfothion (**8**) from 1.00 at 20°C to 1.21 at 40°C. The enantioselective separation of several of the OPs was particularly sensitive to the concentration of the polar modifier. For example, resolution of profenofos (**9**) and trichloronate (**12**) improved from partial resolution to baseline separation when the polar modifier (EtOH) was reduced to 0.5 and 0.0%, respectively.

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Disclaimer: This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the U.S. EPA.

References

[1] E.L. Eliel, S.H. Wilen, Stereochemistry of Organic Compounds, J. Wiley, New York, 1994.
[2] O.P. Rodriguez, G.W. Muth, C.E. Berkman, K. Kim, C.M. Thompson, Bull. Environ.Contam. Toxicol. 58 (1997) 171.

[3] L.P.A. de Jong, H.P. Benschop, in: Proceedings of Third International Meeting on Cholinesterases, American Chemical Society, Washington, DC, 1991, p. 240.
[4] A. Miyazaki, T. Nakamura, M. Kawaradani, S. Marumo, J. Agric. Food Chem. 36 (1988) 835.
[5] D.L. Lewis, A.W. Garrison, K.E. Wommack, A. Whittemore, P. Steudler, J. Melillo, Nature 401 (1999) 898.
[6] N. Ôi, M. Horiba, H. Kitahara, Agric. Biol. Chem. 43 (1979) 24030.
[7] H.E.T. Spruit, H.C. Trap, J.P. Langenberg, H.P. Benschop, J. Anal. Toxicol. 25 (2001) 57.
[8] W. Zhu, F. Wu, F.M. Raushel, Gy. Vigh, J. Chromatogr. A 895 (2000) 247.
[9] C.E. Kientz, J.P. Langenberg, G.J. de Jong, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 14 (1991) 460.
[10] T. Shibata, Poster 2nd International Symposium on Chiral Separartions, September 1989, Guilford, UK.
[11] C.J. Welch, T. Szczerba, Enantiomer 3 (1998) 37.
[12] G.W. Gorder, O. Kirino, A. Hirashima, J.E. Casida, J. Agric. Food Chem. 34 (1986) 941.
[13] E. Yashima, J. Chromatogr. A 906 (2001) 105.
[14] Y. Okamoto, E. Yashima, Angew. Chem. Int. Ed. 37 (1998) 1020.
[15] Y. Okamoto, Y. Kaida, J. Chromatogr. A 666 (1994) 403.
[16] T. Wang, Y.W. Chen, J. Chromatogr. A 855 (1999) 411.
[17] J. Dingenen, A Practical Approach to Chiral Separations by Liquid Chromatography, VCH, Weinheim, 1994.
[18] K. Tachibana, A. Ohnishi, J. Chromatogr. A 906 (2001) 127.
[19] T. O'Brien, L. Crocker, R. Thompson, K. Thompsom, P. Toma, D. Conlon, B. Feibush, C. Moeder, G. Bicker, N. Grinberg, Anal. Chem. 69 (1997) 1999.
[20] E. Küsters, V. Loux, E. Schmid, J. Chromatogr. A 666 (1994) 421.
[21] E. Küsters, C. Spöndlin, J. Chromatogr. A 737 (1996) 333.