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# Evaluation of two commercial capillary columns for the enantioselective gas chromatographic separation of organophosphorus pesticides

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**Abstract**

The separation of the enantiomers of 13 organophosphorus pesticides (OPPs) has been investigated by gas chromatography (GC) with flame ionisation detection (FID) using two different commercially available chiral columns, Chirasil-Val (L-valine-*tert*-butylamide) and CP-Chirasil-Dex CB (heptakis (2,3,6-tri-*O*-metil)-β-cyclodextrin). Using the Chirasil-Val column no chiral resolution was obtained for the OPPs investigated under any tested experimental condition. The use of the CP-Chirasil-Dex CB stationary phase enabled good individual enantiomeric separation of two OPPs, ruelene and trichlorfon and partial separation of naled, chloretoxyphos, isophenphos and metamidophos. Also, the obtained chromatographic results showed that Chirasil-Dex could resolve enantiomers through the combination of different mechanism (e.g. formation of inclusion complexes and/or interactions outside the cyclodextrin cavity).

Under optimised conditions, precision, linearity range and detection limits were evaluated for the enantiomers of ruelene and trichlorfon using CP-Chirasil-Dex CB column and electron capture detection (ECD). By using the GC-ECD method the enantiomers of these OPPs could be satisfactorily detected at very low concentration levels. The detection limits observed were 1.5 ng $mL^{-1}$ and 11.5 ng $mL^{-1}$ for the enantiomers of trichlorfon and ruelene, respectively.


*Keywords:* Enantioselective gas chromatographic separation; Organophosphorus pesticides; GC-ECD; Chirasil-Val; Chirasil-Dex

## 1. Introduction

The development of analytical methods for the efficient separation of enantiomers is one of the most important tasks in several research fields, particularly in pharmaceutical and agrochemical research. This is mainly due to the different biological activity that can be exhibited by a pair of enantiomers. Most published papers have focused on the separation of chiral pharmaceutical products as a consequence of the more severe guidelines for marketing new chiral drugs. However, it should be recognized that the same chiral principles apply to pesticides containing stereogenic centers, e.g. in many organophosphorus compounds.

Organophosphorus pesticides (OPPs) are among the most widely employed world-wide. Their large use, especially for crop protection, may result in the presence of residues of these substances in a wide range of surface and ground waters, drinking waters, fruits, vegetables and foodstuff in general [1–4]. The OPPs act as pesticides because of their property of inhibiting acetylcholinesterase (AchE) in insects. Although these compounds show preferential toxicity to insects, they are also toxic to mammals by the same mode of action [5]. Regulatory limits for human exposure are based on inhibition of AchE either in experimental animals or in humans [6,7]. Moreover, some OPPs have been shown to produce long term neurological damage known as organophosphate-induced delayed polyneuropathy (OPIDP) [5].

Numerous OPPs are enantiomeric compounds with phosphorus or carbon atoms as chiral centers. They are introduced into the environment as racemic mixtures despite that the pesticidal activity is usually due to the preferential reactivity of one enantiomer, while the other may have toxic effects on nontarget organisms [8,9]. Additionally, the microbial degradation and the metabolic pathways of chiral pesticides can be stereoselective [10]. Thus, it would be desirable to use single enantiomers as pesticides.

In order to evaluate the real environmental toxicity of chiral pesticides, to understand their chiral discrimination in biolog-

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ical processes and to assess the enantiopurity of commercial pesticide formulations, analytical separation methods of high stereoselectivity are required. Such enantioselective methodologies will be also useful to control the industrial production of enantiomerically pure agrochemicals.

In this regard, the enantiomeric separation of a limited number of OPPs has been performed mainly by high-performance liquid chromatography (HPLC) [8,10–14] and capillary electrophoresis (CE) [9,15–17]. There are very few publications concerning the use of gas chromatography (GC) in the enantiomeric separation of OPPs [18,19], in spite of the high efficiency and sensitivity advantages of enantioselective GC [20]. Many existing chiral OPPs have not been enantiomerically separated yet by GC, specially using commercial chiral stationary phases (CSPs).

Enantiomeric separation by GC is still based on an empirical selection of the stationary phase. In fact, at present it is not possible to predict the exact "enantiomer-selective" behaviour of a particular stationary phase. Thus, most practical chiral analysis depends on the experience obtained in a case by case basis.

In this line, the aim of this work was to carry out a systematic analytical study of the enantioselective gas chromatographic separation of several chiral OPPs, of present importance and with different structures, via the use of two commercially available chiral stationary phases. The two CSPs selected have different separation mechanisms based on hydrogen-bonding (Chirasil-Val column) [20] and (inter alia) inclusion (CP-Chirasil-Dex CB column) interactions [20]. The results obtained could be of interest to select and adequate CSP for eventual application to the determination of this particular racemic OPPS in real samples. Moreover, the GC determination of those two chiral OPPs separating completely on the CP-Chirasil-Dex CB column has been also investigated here.

## 2. Experimental

### 2.1. Chemicals

The chiral organophosphorus pesticide standards selected in this work: naled, metamidophos, trichlorfon, malaoxon, malathion, isofenphos, phentoate, phenamiphos, leptophos and dialiphos were purchased from Riedel de Haën (Seelze, Germany) while chloretoxyphos, ruelene and pyraclophos were obtained from Chem Service (New Haven, CT, USA). All pesticides were only available as racemates. The pesticides can be divided in two groups, one group containing a carbon atom as chiral center (Fig. 1) and the other group with a stereogenic phosphorus atom (Fig. 2). Stock standard solutions of each OPP at a concentration of 5000 μg $mL^{-1}$ were prepared in *n*-hexane or acetone and stored at −20 °C. These solutions were used for the preparation of different working standard racemic mixtures of the OPPs in *n*-hexane. Acetone and *n*-hexane for pesticides residue analysis were supplied by Riedel de Haën and methanol for ultratrace analysis was obtained from Merck (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

### 2.2. Instrumentation

Gas chromatography was carried out with a HP-5890 Series II gas chromatograph equipped with an electron capture detector (GC-ECD) and a flame ionisation detector (GC-FID) (Hewlett-Packard, Avondale, PA, USA). Helium was used as carrier gas at a pressure of 10 psi. Split/Splitless injections (splitless time 2 min) of 1 μL were carried out. An injector temperature of 260 °C (200 °C in the case of the trichlorfon separation) and a detector temperature (ECD and FID) of 300 °C were used.

Enantiomeric separations were investigated using two chiral columns. The first one, a Chirasil-Val column (25 m × 0.25 mm I.D., Macherey-Nagel, Düren, Germany) with a stationary phase consisted in L-valine-*tert*-butylamide directly bonded to dimethylpolysiloxane; this column has a maximum temperature of 190–200 °C. The second chiral column used was a CP-Chirasil-Dex CB, (25 m × 0.25 mm I.D. × 0.25 μm film thickness, Varian, Walnut Creek, CA) which has a maximum temperature of 200–225 °C. The stationary phase of this column consisted in a heptakis (2,3,6-tri-*O*-metil)-β-cyclodextrin molecule directly bonded to dimethylpolysiloxane.

## 3. Results and discussion

As it was mentioned before, the selection of a suitable GC stationary phase which can undergo enantioselective interactions with the desired enantiomers is still highly empirical. Thus, most practical chiral analysis depends on the experience obtained in a case by case basis. In this work, two commercially available chiral columns were selected to investigate the separation of a wide range of chiral OPPs containing carbon (Fig. 1) or phosphorus (Fig. 2) as the sole asymmetric atom.

### 3.1. Enantiomeric separation of OPPs on a Chirasil-Val column

The Chirasil-Val column was first selected because this stationary phase has excellent properties with respect to thermal stability and separating power for enantiomers able to form hydrogen-bonding interactions. In fact, the enantiomers of some organophosphoroamidothioates have been separated in a column with a similar separation mechanism [21]. Therefore, the GC resolution of only the chiral OPPs containing phosphoroamidate (ruelene, phenamiphos and metamidophos), phosphonate (trichlorfon) or phosphorothioate (malathion and malaoxon) groups in their structures (see Figs. 1 and 2) were attempted with the Chirasil-Val column. Detection was carried out by flame ionisation detector.

Enantioseparations of chiral compounds in gas chromatography are usually governed by enthalpy control and so, the best chiral resolution can be obtained with isothermal elution at low temperatures or slow GC heating rate [20]. Thus, the separation was carried out under isothermal elution at temperatures of 190 °C, 160 °C and 140 °C. The following temperature programs: 50 °C for 1 min, then raised to 190 °C at a rate of 1–4 °C $min^{-1}$ were also assayed. Unfortunately, using the above