Journal of Chromatography A, 1218 (2011) 6561–6582

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Review

Chiral separation of agricultural fungicides

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ARTICLE INFO

Article history:
Received 11 May 2011
Received in revised form 21 July 2011
Accepted 26 July 2011
Available online 3 August 2011

Keywords:
Fungicides
Chiral separation
Capillary electrophoresis
Gas chromatography
High performance liquid chromatography
Supercritical fluid chromatography

ABSTRACT

Fungicides are very important and diverse environmental and agricultural concern species. Their determination in commercial formulations or environmental matrices, requires highly efficient, selective and sensitive methods. A significant number of these chemicals are chiral with the activity residing usually in one of the enantiomers. The different toxicological and degradation behavior observed in many cases for fungicide enantiomers, results in the need to investigate them separately. For this purpose, separation techniques such as GC, HPLC, supercritical fluid chromatography (SFC) and CE have widely been employed although, at present, HPLC still dominates chromatographic chiral analysis of fungicides. This review covers the literature concerning the enantiomeric separation of fungicides usually employed in agriculture grouping the chiral separation methodologies developed for their analysis in environmental, biological, and food samples.

Contents

1. Introduction

The main aim of all research studies about new agricultural products, is the development of new compounds able to offer the same effectiveness with lower application rates, higher selectivity, less cost and less undesired effects [1]. Approximately a 25% of all the agrochemicals used in the world are chiral and this property can have influence on all these aspects due to the different activity that stereoisomers may have [2]. In fact, although stereoisomers have exactly the same physical and chemical characteristics, when exposed to a chiral environment they use to behave very different and this can lead to different activities, toxicology or degradation patterns. If this is the case, when racemic mixtures are employed, from a 50 to a 75% of the amount added into the environment corresponds to an undesired and unneeded compound which may be less active as pesticide or even be toxic against non-target organisms [3]. In spite of this behavior, the most part of these

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Abbreviations: BGE, background electrolyte; CD, cyclodextrin; CD-EKC, cyclodextrin modified electrokinetic chromatography; CD-MEKC, cyclodextrin modified micellar electrokinetic chromatography; CDMPC, cellulose-tris-(3,5-dimethylphenylcarbamate); CM-γ-CD, carboxymethyl-γ-cyclodextrin; CSP, chiral stationary phase; CTB, cellulose tribenzoate; CTMB, cellulose-tris-(4-methylbenzoate); CTPC, cellulose tryphenylcarbamate; DS, degree of substitution; ECD, electron capture detector; EKC, electrokinetic chromatography; EOF, electroosmotic flow; HE-β-CD, hydroxyethyl-β-cyclodextrin; HP-β-CD, hydroxypropyl-β-cyclodextrin; HP-γ-CD, hydroxypropyl-γ-cyclodextrin; HSA, human seroalbumin; LC50, median lethal concentration; MEKC, micellar electrokinetic chromatography; MSPD, matrix solid phase dispersion; SBE-β-CD, sulpho-butyl-ether-β-cyclodextrin; SC, sodium cholate; SDS, sodium dodecyl sulphate; SFC, supercritical fluid chromatography; SPE, solid phase extraction; SRMM, stacking with reverse migrating micelles; Succ-β-CD, succinyl-β-cyclodextrin; Succ-γ-CD, succinyl-γ-cyclodextrin.

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doi:10.1016/j.chroma.2011.07.084

products are marketed and employed as racemates although the total amount of compound released to the environment could be greatly reduced if enantiomerically enriched products were used [4]. The different activity of pesticide enantiomers and the use of pure enantiomers as agrochemicals makes necessary their stereoselective determination in order to optimize enantioselective production processes, assessing the enantiopurity of formulations and monitoring their presence in the environment or into different types of matrices.

Fungicides are chemical compounds or biological organisms used to kill or inhibit fungi or fungal spores. Taking into account that the amount of pesticides employed per year is increasing, the amount of fungicides released to the environment increases at the same rate [5].

There is a great number of types of fungicides. They can be classified according to different criteria but perhaps the most usually employed are (1) mode of action and (2) chemical group [6]. Although some fungicides are chiral there is not much information concerning the role that chirality plays in their properties. Conazole and amide type fungicides are the best documented and there are some works showing the clear differences between the behavior of their enantiomers. Regarding the pesticide activity there are many compounds for which this property is only or mainly associated to one enantiomer. For example, for conazole type fungicides, the R-enantiomer of diniconazole and uniconazole, shows stronger fungicidal activity than the S-enantiomer, whereas the latter has higher plant growth regulating activity [7,8]. The same behavior has been observed for tebuconazole [9] and dichlopentezol whose R-enantiomer is over one hundred times more active as fungicide than the S-enantiomer, but the latter possesses very marked plant growth regulatory properties [10]. Another example is paclobutrazol since its fungicide activity is connected with 2R,3R-paclobutrazol while 2S,3S-paclobutrazol is responsible for the anti-gibberellin activity in plant growth regulation [11]. For triadimenol, a fungicide that possesses four stereoisomers, the (−)-threo-1S,2R-enantiomer shows the highest fungitoxicity against *Rhizoctonia solani* among the four enantiomers [12]. In the case of amide type fungicides most information found in the literature refers enantiomers of metalaxyl and benalaxyl. All the fungicidal activity of these compounds lies in the (+)-enantiomer or R-enantiomer [13].

When biological processes take place, the degradation patterns of each enantiomer may also differ a lot, resulting for example in the higher persistence of the non-active enantiomers. Furthermore, the toxicity against non-target organisms may also be different. For example, tebuconazole is degraded in rabbits following a pseudo-first-order kinetics and the degradation of the S-tebuconazole was much faster than that of the R-enantiomer in plasma after administration of the racemic compound [14]. Enzymatic reactions in rat microsomal material show that under physiological conditions of rat, the *trans*-isomer of bromuconazole reacts faster than the *cis*-isomer. Moreover, *trans*-bromuconazole was found to undergo stereoselective metabolism as evidenced by a change in the enantiomeric ratio (*trans* (−)/*trans* (+)) with time [15]. It was also reported that (−)-imazalil was degraded more quickly than (+)-imazalil in oranges [16], however, when imazalil was analyzed in soil extracts the degradation observed for both enantiomers was just the same. Due to the fact that the pH of the soils analyzed in this study was slightly alkaline, it is possible that the microbial activity under these conditions was limited and thus the degradation was not enantioselective [17]. The degradation of metalaxyl in most type of soils was shown to be enantioselective with the fungicidal active enantiomer, R-metalaxyl being degraded faster than the inactive S-enantiomer, resulting in residues enriched with S-metalaxyl when the racemic compound was incubated [13]. However, the S-enantiomer of this compound showed a faster degradation in plants [18] and in rabbits, where its levels in plasma, liver and kidney decreased more rapidly than for the R-enantiomer [19]. Furthermore, acute toxicities of racemic metalaxyl and R-metalaxyl to *Daphnia magna* were determined and compared by Chen et al. [20]. The median lethal concentration (LC50) values for racemic and R-metalaxyl to *D. magna* showed the clear difference in the toxicity of the two enantiomers against this organism. In the case of benalaxyl, several articles have reported the differences in degradation between its two enantiomers in tomato, tobacco, sugar beet, capsicum, cucumber, and some kind of soils [21,22]. The results in soil showed that R-enantiomer dissipated faster than S-enantiomer and the stereoselectivity might be caused by microorganisms [21,22]. In tobacco, tomato, sugar beet and capsicum plants, there was significantly stereoselective metabolism. In effect, the preferential absorption and degradation of S-benalaxyl resulted in an enrichment of the R-enantiomer residue in these vegetables [21]. In terms of enantiotoxicity, for earthworm in paper contact test, after 48 h of exposure, the calculated LC50 values of the R-form and S-form were 4.99 and 6.66 μg/cm^2, respectively, demonstrating the clear differences in the toxicity of the two enantiomers of benalaxyl against this non-target organism [23]. Finally, vinclozolin (dicarboximide fungicide) has been proved to be enantioselectively degraded in wines during wine-making process, resulting in the enrichment of (−)-vinclozolin [24].

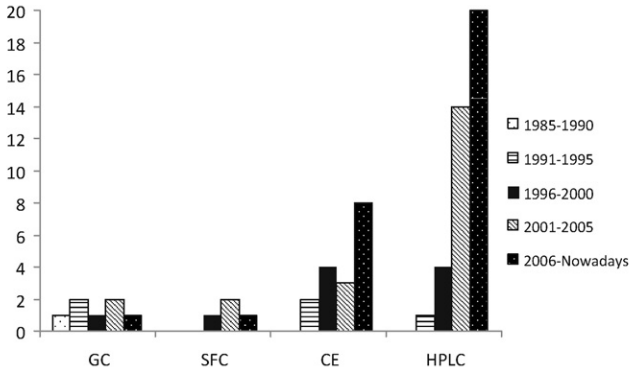

Fig. 1. Graphic showing the number of publications per year of each technique in the field of chiral separation of fungicides.

Taking into account all mentioned, chiral separation techniques with high efficiency are needed to determine the stereoisomers of pesticides in environmental and other many matrices and to understand the enantiomeric discrimination in these media in order to improve both our understanding of the pesticides and safety to humans, animals and environment [25].

Chiral separation of fungicides has been performed by different techniques (GC, HPLC, SFC, and CE). A chiral separation by these analytical techniques can be performed by two different experimental approaches: indirect or direct separations. Indirect separation needs a previous derivatization step with a chiral agent in order to transform the enantiomers into diastereoisomers that can be easily separated into an achiral column. To achieve the chiral separation successfully a nearly 100% pure enantiomeric derivatization reagent is needed [26]. Only a work is described in the literature using this strategy [27], being the direct separation of fungicide enantiomers employed in most cases. This strategy involves the addition of a chiral selector to the separation media or the employ of CSPs. The formation of reversible diastereoisomeric complexes is the responsible for the chiral discrimination [28]. As it can be observed in Fig. 1, the most employed technique for the determination of fungicide enantiomers is HPLC. In fact, the wide variety of chiral columns able to resolve a large number of fungicides has made HPLC a versatile technique widely applied to the determination of fungicides at low concentration levels. UV detec-