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Chiral separation of some triazole pesticides by supercritical fluid chromatography☆

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Abstract

The enantiomeric separation of six triazole pesticides: cyproconazole, propiconazole, diniconazole, hexaconazole, tebuconazole and tetraconazole, using supercritical fluid chromatography and the Chiralpak AD column, is presented in this work. The effect of different organic modifiers such as methanol, ethanol and 2-propanol on the retention and resolution was studied as well as the presence of additives in the mobile phase. The results obtained were highly successful, all the compounds were enantiomerically separated and in most of the cases the analysis time was close to 10 min. The type of organic modifier that provided the best results depended on the compound.

Keywords: Enantiomer separation; Supercritical fluid chromatography; Pesticides; Triazoles

1. Introduction

The development of analytical methods for the separation of enantiomers is one of the most important tasks in several research fields such as pharmaceutical or agrochemical, since it is well known that a pair of enantiomers can display different biological activity. Although most of the published papers focused on the separation of chiral pharmaceuticals products, as a consequence of the more severe guidelines for marketing new chiral drugs, it should be recognized that the same principles are important for pesticides containing stereogenic centers. In this way, a better knowledge of the individual degradation or toxicological data of each enantiomer could reduce the amount of pesticide used and avoid the unnecessary stereoisomer.

Today's enantiomeric separations are mostly carried out using high-performance liquid chromatography (HPLC) [1–3] and among the different chiral stationary phases (CSPs) marketed, the Chiralpak or the Chiralcel are the most commonly used, due to the high number of successful separations obtained [4–9]. Nevertheless, in the last few years, supercritical fluid chromatography (SFC) has emerged as a powerful tool for chiral separations, showing several advantages over HPLC [5,9–11]. The physico-chemical properties of the supercritical fluids allow to obtain separations with high efficiencies and short analysis times, moreover the analysis of polar or thermolabile compounds is possible.

Triazole pesticides are one of the most important families of fungicides due to their great activity against a wide-spectrum of crop diseases. Most of them have stereogenic centers and they consist of one or two pairs of enantiomers, which can lead to important consequences regarding their bioactivity. For instance, in the case of triadimenol it is known that the biological response of each enantiomer is different [12]. Nevertheless, most of the triazole pesticides, with the exception of uniconazole and diniconazole, are comercialized as racemic mixtures, thus enantiomeric methods of analysis are needed not only to make a correct determination of their biological activity, but also to obtain small quantities of each enantiomer from a semipreparative point of view.

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The enantiomeric separation of triazole pesticides has been performed using principally HPLC on different chiral stationary phases such as urea type [13] and polysaccharide derivatives [14,15]. Micellar electrokinetic chromatography (MEKC) [16–18] and capillary electrophoresis (CE) [19,20] using modified cyclodextrines have also been employed, but there are very few publications concerning the use of SFC in the enantiomeric separation of triazole pesticides. In a previous work, we studied the chiral separation triadimenol and triadimefon [21] using SFC on the Chiralpak AD column obtaining very good results in terms of analysis time and resolution. For this reason, the aim of this work was to study the capabilities of SFC on the Chiralpak AD column for the enantiomeric separation of six chiral triazole pesticides.

2. Experimental

2.1. Chemicals

The compounds studied (Fig. 1) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The purity level was higher than 98% and all of them were in their racemic form, except for diniconazole which was predominantly in its *R*-stereoisomeric conformation. The stock solutions of the individual fungicides were prepared in acetonitrile at the 100 mg/l level. The organic solvents used were of HPLC grade and obtained from Lab-Scan (Dublin, Ireland). Carbon dioxide was of SFC-grade and purchased from Carburos Metálicos (Barcelona, Spain).

2.2. Instrumentation

The supercritical fluid chromatograph used was an HP 1205A model from Hewlett-Packard (Wilmington, DE, USA) equipped with a diode-array detector (DAD) and a Rheodyne 7410 injector of 20 μl loop volume (Cotati, CA USA). Detection was made at 220 nm. The system was controlled from the HP-SFC ChemStation Rev.A.01.02. The chiral column employed, a Chiralpak AD, 250 mm × 4.6 mm, packed with the 3,5-dimethylphenylcarbamate derivative of amylose, coated on a 10-μm silica-gel support, was obtained from Daicel (Deventer, The Netherlands).

3. Results and discussion

The compounds studied (Fig. 1) belong to the triazole fungicide family and consist of two enantiomers, except for cyproconazole and propiconazole which have two chiral

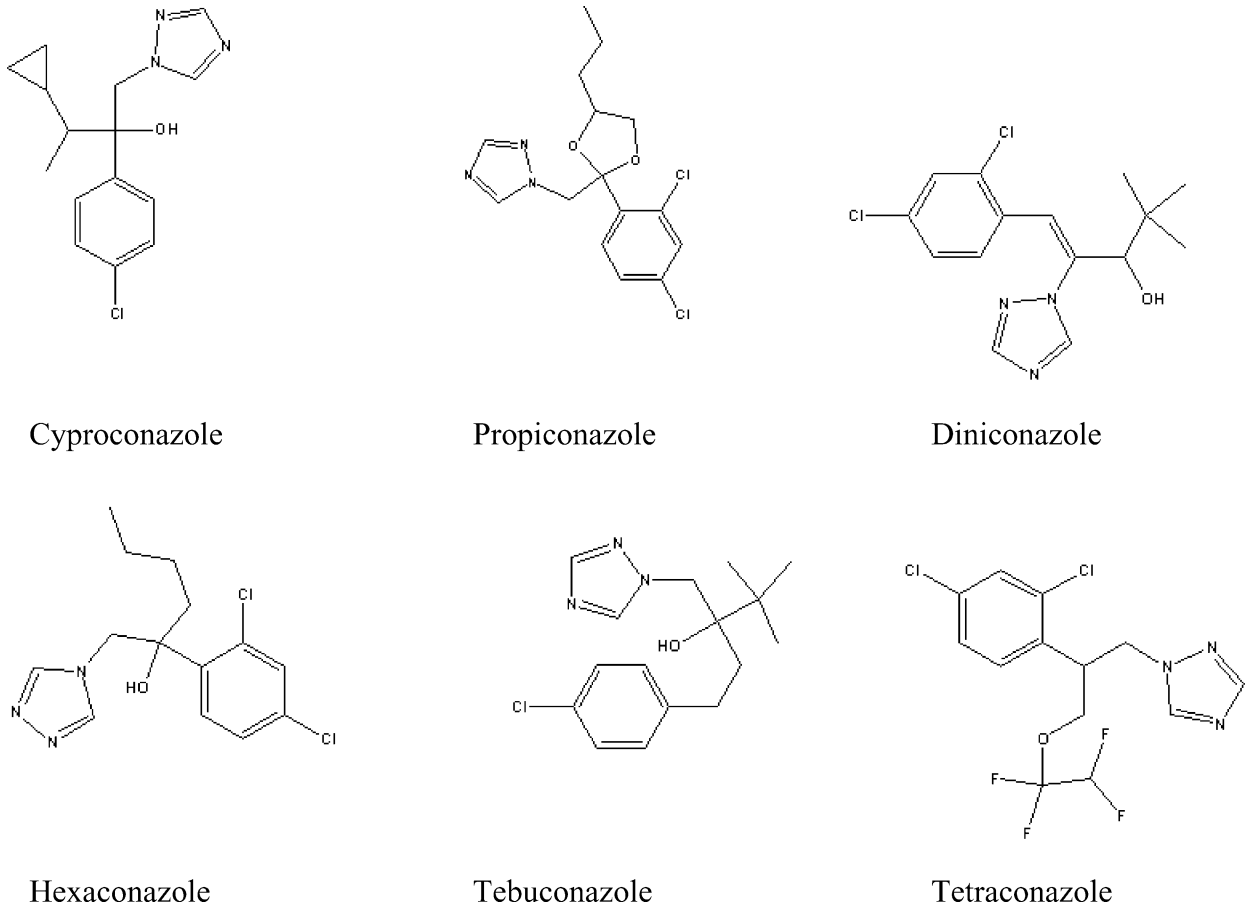

Fig. 1. Structure of the compounds studied.

Table 1
Values of capacity factors and resolutions obtained for cyproconazole and propiconazole using different organic modifiers

Compound	k_1	k_2	k_3	k_4	$R_{s,1-2}$	$R_{s,2-3}$	$R_{s,3-4}$
Cyproconazole							
Methanol (%, v/v)							
5	11.99	13.53	14.65	18.50	1.49	1.01	2.83
10	4.96	5.97	5.97	8.33	2.20	0	3.95
15	2.97	3.59	3.59	5.13	2.07	0	4.10
Ethanol (%, v/v)							
5	14.92	14.92	17.57	22.61	0	2.14	3.76
10	4.94	4.94	5.82	8.02	0	1.71	4.23
15	2.62	2.62	3.04	4.27	0	1.35	4.09
2-Propanol (%, v/v)							
5	12.60	12.60	12.60	15.35	0	0	2.80
10	5.86	5.86	5.86	7.22	0	0	2.00
15	2.39	2.39	2.59	3.27	0	0.81	2.57
Propiconazole							
Methanol (%, v/v)							
3	6.37	6.37	7.21	7.91	0	1.30	1.14
5	3.0	3.0	3.48	3.97	0	1.79	1.69
Ethanol (%, v/v)							
3	5.81	6.48	7.09	8.28	1.51	1.37	2.26
5	2.66	2.93	3.26	3.74	1.13	1.31	1.74
2-Propanol (%, v/v)							
3	11.13	15.18	19.01	23.06	4.03	3.10	2.68
5	5.37	7.51	9.07	11.37	4.26	2.59	3.24

centers and therefore, they consist of four enantiomers. All of them have a common structural moiety: the 1,2,4-triazole ring, and several functional groups that can interact with the stationary phase. As a consequence, the use of an organic modifier was necessary in all the cases to reduce the retention and to obtain reasonable analysis times.

The influence of three different organic modifiers, methanol, ethanol and 2-propanol, on the separation was studied at a temperature of 35 °C, a flow-rate of 2 ml/min and a pressure of 200 bar.

As it can be seen in Tables 1 and 2, all the compounds could be enantiomerically resolved using SFC, and the organic modifier that provided the best results depended on the compound. As a general tendency, the retention increased from methanol to 2-propanol, but in the case of cyproconazole and hexaconazole the retention time decreased using 2-propanol.

As far as resolution is concerned, the biggest variations were obtained by changing the nature of the organic modifier. In the case of cyproconazole, the enantiomeric separation of the four stereoisomers could only be achieved using 5% (v/v) of methanol (Fig. 2). The presence of additives (0.1% of triethylamine and 0.1% of trifluoroacetic acid), in the mobile phase did not improve the separation and using the other organic modifiers, a pair of stereoisomers coeluted, obtaining always chromatograms with three peaks. On the contrary, propiconazole, the other compound that consisted of four enantiomers, was resolved using 3% (v/v) of ethanol or 5% (v/v) of 2-propanol. In this case 2-propanol provided the

Table 2
Values of capacity factors and resolutions obtained for diniconazole, tetraconazole, hexaconazole and tebuconazole using different modifiers

Compound	k_1	k_2	R_s
Hexaconazole			
Methanol (%, v/v)			
5	5.35	5.68	0.67
10	2.33	2.59	1.02
15	1.43	1.61	0.87
Ethanol (%, v/v)			
5	5.14	6.23	2.41
10	2.01	2.28	1.14
15	1.19	1.32	0.65
2-Propanol (%, v/v)			
5	4.38	5.01	0.54
10	2.71	2.90	0.48
15	1.45	1.45	0
Tebuconazole			
Methanol (%, v/v)			
5	13.23	14.74	1.28
10	5.26	5.82	1.17
15	3.11	3.42	1.03
Ethanol (%, v/v)			
5	7.16	7.16	0
10	5.04	5.04	0
15	2.69	2.69	0
2-Propanol (%, v/v)			
5	20.36	22.53	0.98
10	7.61	8.35	1.20
15	3.54	3.91	1.15
Tetraconazole			
Methanol (%, v/v)			
1	4.30	5.23	1.55
3	2.05	2.41	1.27
4	1.06	1.83	1.02
Ethanol (%, v/v)			
1	4.26	5.29	3.05
3	2.24	2.84	2.49
4	1.68	1.98	2.06
2-Propanol (%, v/v)			
1	5.86	8.75	4.98
4	3.28	5.46	4.01
5	2.40	3.69	3.43
Diniconazole			
Methanol (%, v/v)			
5	7.29	12.44	6.08
10	2.81	5.69	6.90
15	1.63	3.43	5.87
Ethanol (%. v/v)			
5	6.97	10.63	4.55
10	2.53	3.87	3.77
15	1.37	2.08	3.11
2-Propanol (%, v/v)			
5	12.46	21.06	1.23
10	4.09	4.09	0
15	2.04	2.04	0

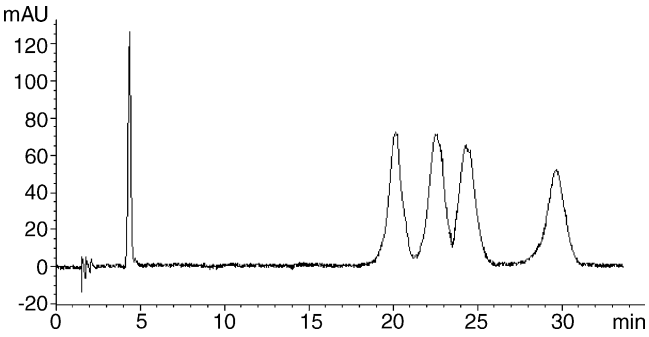

Fig. 2. Separation of cyproconazole stereoisomers. 200 bar, 35 °C, 2 ml/min and 5% (v/v) methanol.

highest resolutions, which allowed using flow-rates as high as 4 ml/min maintaining the baseline resolution and reducing the analysis time to 10 min (Fig. 3).

The baseline enantioseparation of hexaconazole enantiomers was obtained using 5% (v/v) of ethanol. As the peaks were tailed, the inclusion of additives (0.1% triethylamine and 0.1% trifluoroacetic acid) was considered and better peak shapes were achieved. Nevertheless, as it is shown in Fig. 4, the use of additives had a higher effect on the separation when 2-propanol was employed. In this case, the resolution increased from 0.48 to 4.35. The presence of additives was also investigated in the enantioseparation of tebuconazole. The results listed in Table 2, without using additives, show that tebuconazole could not be baseline resolved, but using 10% (v/v) of methanol containing 0.1% of the additives, the resolution obtained was 1.44 (Fig. 5).

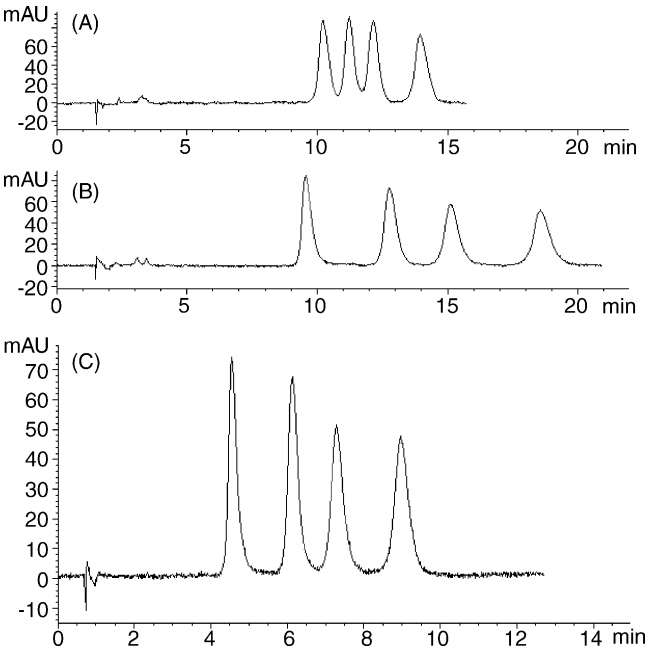

Fig. 3. Separation of propiconazole stereoisomers at 200 bar, 35 °C, 2 ml/min. (A) 3% (v/v) Ethanol; (B) 5% (v/v) 2-propanol; (C) 4 ml/min and 5% (v/v) 2-propanol.

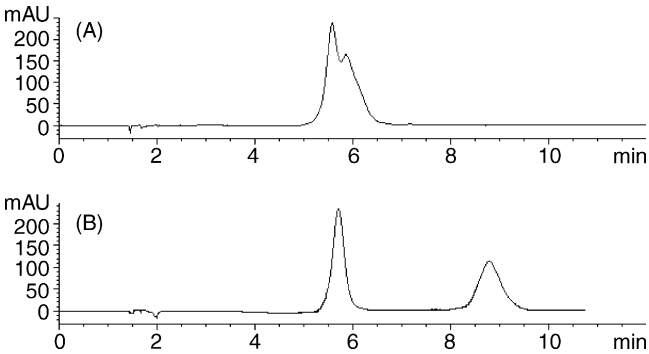

Fig. 4. Enantiomeric separation of hexaconazole at 200 bar, 35 °C, 2 ml/min and 10% (v/v) 2-propanol. (A) Without using additives. (B) Using 0.1% (v/v) triethylamine and 0.1% (v/v) trifluoroacetic acid.

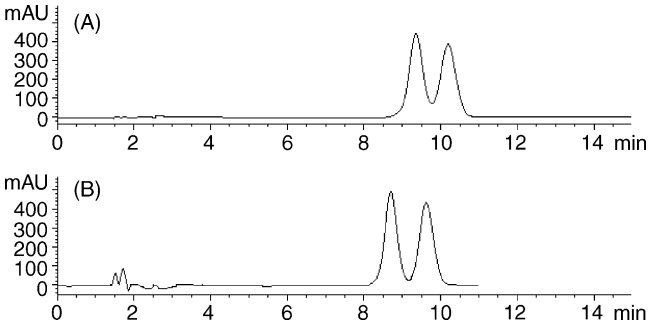

Fig. 5. Enantiomeric separation of tebuconazole at 200 bar, 35 °C, 2 ml/min (A) 10% (v/v) methanol (B) 10% (v/v) methanol containing 0.1% (v/v) triethylamine and 0.1% (v/v) trifluoroacetic acid.

Tetraconazole presented a weak retention on the stationary phase, so it could be eluted with small percentages of modifier. All the organic solvents assayed provided good resolutions for this compound. The highest resolutions were reached using 2-propanol, but the best results in terms of high resolution and low analysis time were obtained using 4% (v/v) of ethanol. In the case of diniconazole, the enantiomers could be separated using methanol or ethanol as modifier. Methanol provided the highest resolutions, but using 15% (v/v) of ethanol the analysis time was lower

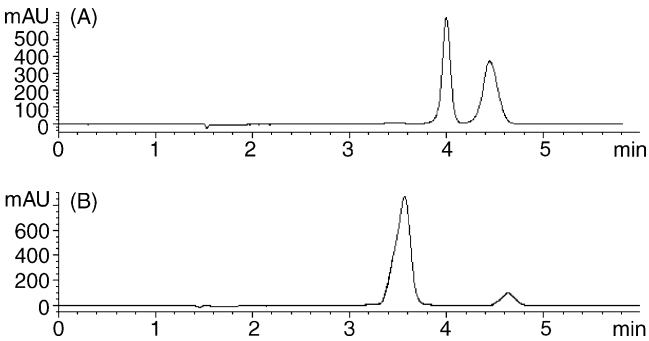

Fig. 6. Enantiomeric separation at 200 bar, 35 °C, 2 ml/min. (A) Tetraconazole 4% (v/v) ethanol; (B) diniconazole 15% (v/v) ethanol.

and the resolution was good enough. In Fig. 6 the chromatograms obtained for tetraconazole and diniconazole are showed.

4. Conclusion

SFC on the Chiralpak AD column is a good technique for the enantiomeric separation of the triazole pesticides studied. The results obtained were highly successful because all the compounds, even those that consisted of four enantiomers, were baseline resolved. The use of an organic modifier or another, depends on the compound to be separated. This way, methanol provided the highest resolutions in the case of diniconazole and cyproconazole, 2-propanol in the case of propiconazole and tetraconazole, and ethanol in the case of hexaconazole. The presence of additives in the mobile phase improved the enantioseparation of hexaconazole using 2-propanol as an organic modifier, and of tebuconazole using methanol.

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