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Separation of triadimefon and triadimenol enantiomers and diastereoisomers by supercritical fluid chromatography

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Abstract

The enantiomeric separation of triadimenol and triadimefon on a Chiralpak AD column using supercritical fluid chromatography, was studied in this work. The effect of different modifiers (methanol, ethanol and 2-propanol) was tested, with methanol and ethanol providing the best results for the enantiomeric separation of the two compounds. The enantioseparation of a mixture of triadimenol and triadimefon (six stereoisomers) was achieved in only 15 min using a gradient of ethanol, 200 bar, 35 °C and a flow-rate of 2 ml/min. The separation of triadimenol diastereoisomers on different achiral columns (diol, silica and ODS) was also investigated. In this case, the type of organic modifier to be used depended on the stationary phase, the Spherex Diol being the column that gave the best separation. Using this column, resolutions higher than 3 were obtained in analysis times of 5 min with any of the modifiers checked.

Keywords: Enantiomer separation; Supercritical fluid chromatography; Triadimefon; Triadimenol

1. Introduction

Chirality plays an important role in biological systems due to the fact that drug–receptor interactions can be stereoselective. This can cause preferential interaction with one enantiomer over the other, and thus, the desired biological activity of a racemic mixture may be limited to only one enantiomer, the activity of the other being less effective, different or absent. Moreover, the processes of degradation can also be clearly diverse.

Pesticides are just one example of chiral compounds. Approximately 25% of the pesticides in use are chiral, and only a small fraction of all are manufactured and used in the form of pure enantiomeric compounds [1], apparently due to economic and/or technical reasons. Nevertheless, ignoring the existence of enantiomers can produce incorrect toxicological, distribution or degradation data. As a consequence, nowadays researchers are focusing on the study of the individual properties of each enantiomer [2–4] and for this purpose enantiomeric methods of analysis are needed.

Chromatographic techniques are by far the most commonly used in enantiomeric separations, and capillary gas chromatography (cGC) is the first choice for pesticide analysis. However, when analyzing polar or thermolabile pesticides, derivatization

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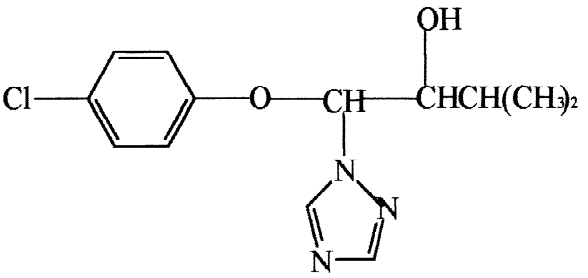
reactions are needed or other chromatographic techniques must be used. In these cases, supercritical fluid chromatography (SFC) is a good alternative. Due to the unique properties of supercritical fluids, high efficiencies and resolutions can be obtained in short analysis times, compared with LC. It also offers the possibility of analysing polar or thermolabile compounds; nowadays the enantioseparation of chiral compounds is the most successful area for SFC separations [5–12].

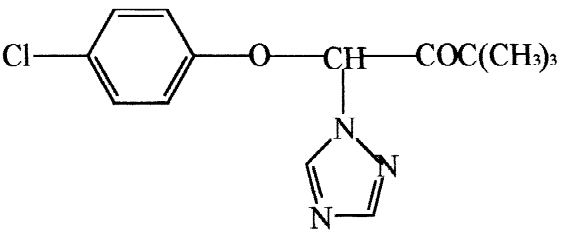
Triadimefon and triadimenol belong to the family of the triazole pesticides and are two of the most important fungicides in use. Both of them have chiral centers, and consist of one and two pairs of enantiomers, respectively. It is known that the biotransformation of triadimefon into triadimenol is stereoselective [13] and the biological response of each triadimenol enantiomer is different, thus enantioselective methods of analysis are necessary in order to make correct determinations of these compounds in studies of their distribution or environmental fate.

The enantiomeric separation of triadimenol and triadimefon has been accomplished using different chromatographic techniques: GC with chiral derivatization [14], HPLC [15], MEKC [16,17] or CE [18,19] always using modified cyclodextrins, but SFC has not been used yet.

The aim of this paper was to check the capability of SFC for the separation of the enantiomers and diastereoisomers of triadimenol and triadimefon. For this purpose, a Chiralpak AD column and several achiral columns were used and the effect of different modifiers was investigated.



Triadimenol



Triadimefon

Fig. 1. Structures of triadimenol and triadimefon.

2. Experimental

2.1. Reagents

Triadimefon and triadimenol (Fig. 1) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The purity level was higher than 98% and triadimenol was predominantly in its *threo*-diastereoisomeric form. The stock solutions were prepared in acetonitrile at the 100 mg/l level. Methanol, absolute ethanol and 2-propanol 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

An HP 1205A model supercritical-fluid chromatograph from Hewlett-Packard (Wilmington, DE, USA) equipped with a diode-array detection (DAD) system and a pneumatically driven injector 7410 Rheodyne (Cotati, CA, USA) valve (5-μl loop volume) was used. Detection was carried out at 220 nm. The instrument was operated in the downstream mode, which means that the pressure is regulated after the column. Pressure was kept constant at 200 bar. The system was controlled from the HP-SFC CHEMSTATION Rev.A.01.02. The chiral column employed, a Chiralpak AD, 250×4.6 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).

The achiral columns used for the separation of triadimenol diastereoisomers were: a Hypersil silica (200×4.6 mm; 5 μm) from Hewlett-Packard (Böblingen, Germany), a Spherclone ODS (250×4.6 mm, 5 μm) and a Spherex diol (250×4.6 mm, 5 μm) both obtained from Phenomenex (Torrance, CA, USA)

2.3. Methods

Based on our previous experience [20–22], this study focused on the influence of the kind and percentage of organic modifier on the separation, fixing the pressure at 200 bar, the flow-rate at 2 ml/min and the temperature at 35 °C.

When gradients of organic modifiers were used, the equilibration time between runs was 6 min. On changing the type of organic modifier, the equilibration time was 15 min.

All the data given in this work are the mean of three consecutive injections. The values of retention factors, selectivity and resolutions were given by the software and calculated according with the following mathematical expressions:

$$k' = (t_R - t_0)/t_0$$

$$\alpha = k'_2/k'_1$$

$$R_s = 2(t_{R2} - t_{R1})/(\omega_1 + \omega_2)$$

where t_0 is the dead time, t_R the retention time of the compound and ω the peakwidth at the base of the peak.

3. Results and discussion

3.1. Separation of triadimenol isomers

3.1.1. Separation of triadimenol stereoisomers

Triadimenol is a triazole fungicide with two chiral centres, so it consists of two diastereoisomers, and each diastereoisomer of two enantiomers, which means four stereoisomers.

As can be seen in Table 1, the separation of the four stereoisomers could be achieved by using methanol or ethanol as organic modifiers, while with 2-propanol only the enantioresolution of the first eluted diastereoisomer was obtained. The effect of increasing the percentage of the organic modifier in the mobile phase was the same for all the modifiers studied: there was a decrease in the retention as a consequence of the increase in the polarity of the mobile phase. Keeping the same percentage of modifier, methanol and ethanol provided similar capacity factors except for the last eluting enantiomer, for which, and opposite to expectations, retention decreased when changing from methanol to ethanol. As far as enantioresolution is concerned, it decreased slightly with increasing the percentage of organic modifier in the mobile phase, but the biggest variations were obtained by changing the modifier.

Table 1
Effect of the modifier on the separation of triadimenol enantiomers

Modifier	k'_1	k'_2	k'_3	k'_4	α_1	α_2	α_3	R_{s1}	R_{s2}	R_{s3}
Methanol										
5%	5.41	6.44	8.11	22.55	1.19	1.26	2.78	1.81	2.47	10.88
10%	1.86	2.22	2.55	8.59	1.19	1.15	3.37	1.45	1.21	11.02
20%	0.64	0.79	0.79	2.98	1.23	1.00	3.77	0.80	0.00	8.76
Ethanol										
8%	2.52	3.63	4.05	8.78	1.44	1.12	2.17	3.86	1.20	8.60
10%	1.82	2.61	2.90	6.27	1.43	1.11	2.16	3.20	0.95	7.67
15%	0.96	1.36	2.96	2.96	1.42	2.17	1.00	2.38	6.83	0.00
20%	0.70	0.98	0.98	2.06	1.40	1.00	2.10	1.42	0.00	5.00
2-Propanol										
10%	3.31	5.76	6.43	6.43	1.74	1.12	1.00	5.25	1.86	0.00
15%	1.49	2.58	2.96	2.96	1.73	1.15	1.00	4.30	1.19	0.00
20%	0.82	1.45	1.45	1.45	1.77	1.00	1.00	3.32	0.00	0.00

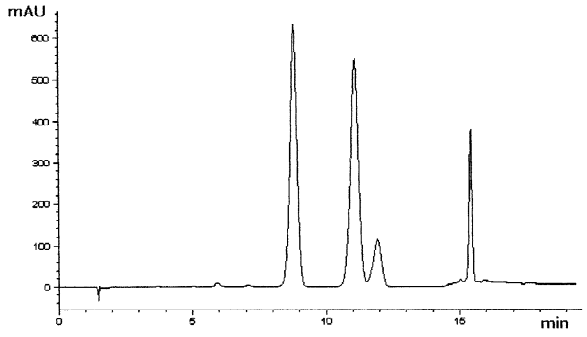


Fig. 2. Separation of triadimenol enantiomers on the Chiralpak AD column using ethanol as modifier and programmed from 5% (2 min) to 25% at 1.8%/min.

The enantioresolution of the first eluting diastereoisomer was higher using ethanol while for the second one it was lower. The best results in terms of resolution ($R_s > 1.5$) and analysis time (<16 min) were obtained by programming a gradient of ethanol from 5% (2 min) to 25% at 1.8%/min (Fig. 2).

3.1.2. Separation of triadimenol diastereoisomers

The separation of triadimenol diastereoisomers was investigated using achiral columns and the results obtained are shown in Table 2. The best separation was obtained with the Spherex Diol column and the worst with the apolar stationary phase ODS. Moreover, the type of organic modifier to be used depended on the stationary phase, as is

Table 2
Effect of the different modifiers and columns on the separation of triadimenol diastereoisomers

Column	Modifier	k'_1	k'_2	α	R_s
Spherclone	Ethanol				
ODS	3%	9.95	10.68	1.07	1.84
	5%	3.39	3.47	1.02	0.81
	8%	1.42	1.42	1.00	0.00
	10%	0.96	0.96	1.00	0.00
	2-Propanol				
	5%	9.69	10.59	1.09	1.02
	8%	3.54	4.04	1.14	1.16
	10%	2.21	2.55	1.15	1.16
	15%	0.97	1.13	1.16	1.12
Spherex	Methanol				
diol	3%	5.90	8.15	1.38	8.53
	5%	2.70	3.63	1.34	6.55
	10%	1.16	1.48	1.27	3.92
	15%	0.71	0.86	1.21	2.44
	Ethanol				
	3%	7.11	9.43	1.33	7.25
	5%	3.60	4.75	1.32	5.70
	10%	1.53	1.95	1.27	4.01
	15%	0.95	1.16	1.22	2.75
	2-Propanol				
	3%	12.23	16.19	1.32	4.69
	5%	5.77	7.66	1.33	5.58
	10%	2.16	4.21	1.95	4.48
	15%	1.22	1.54	1.26	3.45
Hypersil	Methanol				
silica	3%	3.60	4.41	1.23	1.18
	5%	1.78	1.68	0.94	1.35
	10%	0.44	0.52	1.18	0.94
	Ethanol				
	3%	2.61	2.89	1.11	1.12
	5%	1.78	1.96	1.01	1.39
	10%	0.67	0.71	1.06	1.05

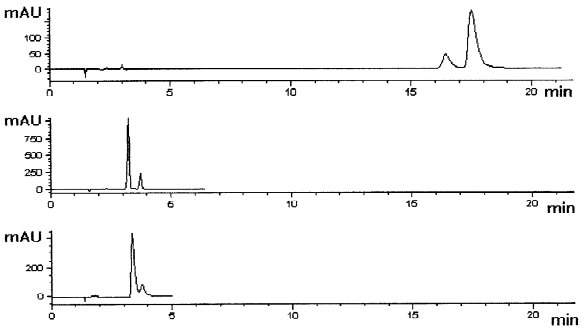


Fig. 3. Separation of triadimenol diastereoisomers using different columns and the best conditions with each one. (A) Spherclone ODS 3% ethanol (B) Spherex Diol 10% methanol (C) Hypersil silica 3% methanol.

Table 3
Effect of the different modifiers on the separation of triadimefon enantiomers

Modifier	k'_1	k'_2	α	R_s
Methanol				
3%	3.98	6.18	1.55	3.02
5%	2.37	4.16	1.76	5.08
10%	1.33	2.48	1.86	4.94
15%	1.03	1.91	1.85	4.09
Ethanol				
3%	5.83	7.28	1.25	2.13
5%	3.14	4.11	1.31	2.94
10%	1.34	1.82	1.36	2.65
15%	0.88	1.21	1.37	1.95
2-Propanol				
5%	6.05	6.18	1.02	0.46
10%	1.63	1.63	1.00	0

shown in Fig. 3. Although the ODS column provided the largest analysis time, it should be noted that there was an inversion of the selectivity. This fact could be an advantage when determining the diastereoisomeric purity, because the minor diastereoisomer eluted before the main diastereoisomer enhancing its detection at low levels (Fig. 3). When the Spherex Diol column was used, good results were obtained with any of the modifiers studied. In this case, the retention increased from methanol to 2-propanol and the resolution decreased in the same way, methanol being the modifier which provided the highest resolution in the lowest analysis time. Using the ODS column the best results were obtained with ethanol; with the use of 2-propanol, although it provided some kind of separation, the peaks were too broad, and employing methanol the peaks coeluted. Finally, with the Hypersil silica column, the most polar stationary phase, methanol and ethanol provided quite similar results in terms of resolution, analysis time and peak width. When using 2-propanol the separation was not achieved any way.

Taking all the results into account, separation of the four triadimenol stereoisomers can be achieved in 15.5 min using the Chiralpak AD column and a gradient of ethanol. The diastereoisomers can be resolved in 4 min on the Spherex Diol column using 10% of methanol.

3.2. Separation of triadimefon enantiomers

Triadimefon consists of two enantiomers whose separation was studied using the Chiralpak AD column. The results obtained are shown in Table 3. Methanol and ethanol provided good results—in both cases resolutions were higher than 1.5 and the ones achieved with methanol were always higher than those with ethanol. When using $<5\%$ of organic modifier, the retention decreased from ethanol to methanol, but using $\geqslant 5\%$ unexpected results were obtained. This was due to the fact that ethanol provided a lower retention than methanol, although it has a lower polarity. Using 2-propanol the enantiomeric separation was more difficult and the baseline separation was obtained programming it from 5% (5 min) to 30% at 5%/min (Fig. 4).

3.3. Simultaneous enantiomeric separation of triadimenol and triadimefon

Triadimenol and triadimefon can occur together, due to the fact that triadimenol is the main metabolite of triadimefon and it has by itself antifungal activity. It is interesting to investigate the simultaneous enantiomeric separation of both compounds on the Chiralpak AD column.

Fig. 5 shows that to obtain baseline separation it was necessary to use gradients of modifier, methanol and ethanol being the solvents that provided the best results. The mixture of the six stereoisomers could be resolved in only 15.5 min using ethanol programmed from 5% (2 min) to 25% at 1.8%/min.

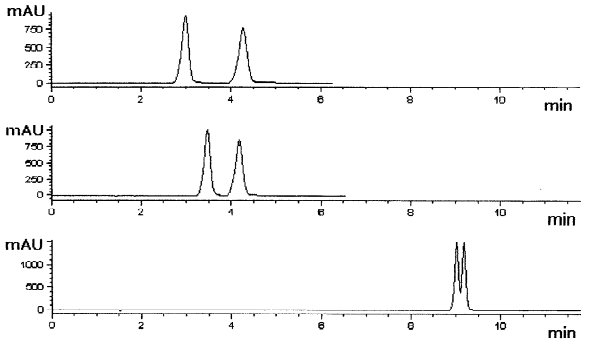


Fig. 4. Separation of triadimefon enantiomers. (A) 15% Methanol; (B) 10% ethanol; (C) 2-propanol programmed from 5% (5 min) to 30% at 5%/min.

4. Conclusions

Triadimenol and triadimefon can be enantiomerically separated on the Chiralpak AD column using SFC. Of all the organic modifiers studied, methanol and ethanol provided the best results always achieving resolutions >2, while 2-propanol failed to separate the enantiomers, especially in the case of triadimenol. For both compounds and opposite to expectations, the retention of the last eluted enantiomer decreased when changing from methanol to ethanol. In all cases, retention decreased when increasing the percentage of a given modifier, while resolution was affected most by changing the type of organic modifier.

SFC on the Chiralpak AD column is a powerful tool for the enantioseparation of a mixture of triadimenol and triadimefon. The six stereoisomers can be separated in only 15.5 min using a gradient of ethanol, 200 bar, 35 °C and a flow-rate of 2 ml/min.

Finally, the separation of triadimenol diastereoisomers can also be accomplished using achiral supercritical fluid chromatography. In this case the best results were obtained with the Spherex Diol column, since whichever of the modifiers used, it provided resolutions >3 in analysis times close to 5 min.

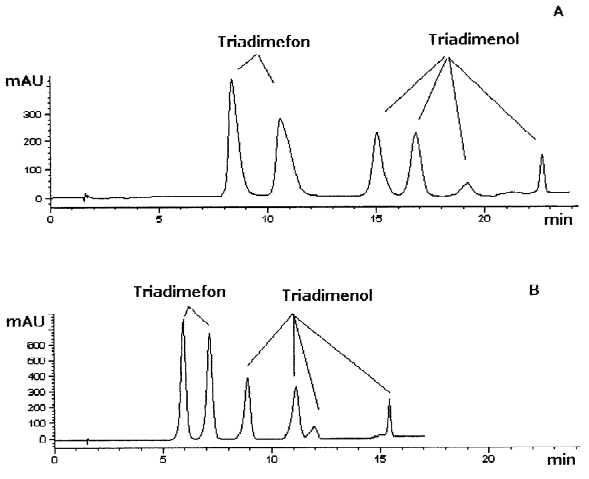


Fig. 5. Simultaneous separation of triadimefon and triadimenol enantiomers. (A) Methanol programmed from 2% (1 min) to 5% at 0.2%/min, held for 3 min and then to 25% at 20%/min. (B) Ethanol programmed from 5% (2 min) to 25% at 1.8%/min.

Acknowledgements

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