Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1046 (2004) 67–73
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Stereoselective determination of allethrin by two-dimensional achiral/chiral liquid chromatography with ultraviolet/circular dichroism detection

F. Mancini[a], J. Fiori[a], C. Bertucci[a], V. Cavrini[a], M. Bragieri[b], M.C. Zanotti[b], A. Liverani[b], V. Borzatta[b], V. Andrisano[a,*]

[a] *Dipartimento di Scienze Farmaceutiche, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy*
[b] *Endura SpA, V.le Pietramellara 5, 40100 Bologna, Italy*

Received 24 March 2004; received in revised form 1 June 2004; accepted 15 June 2004

Dedicated to Dr. Irving W. Wainer on the occasion of his 60th birthday.

Abstract

A two-dimensional achiral/chiral HPLC method with circular dichroism (CD) detection was optimized for the stereochemical resolution and determination of the elution order of the eight stereoisomers of synthetic allethrin. A monolithic silica HPLC column (Chromolith, Merck, 100 mm × 4.6 mm i.d.) was put orthogonally to an enantioselective OJ Daicel column (250 mm × 4.6 mm i.d.) by means of a switching valve. The resolution of *cis* and *trans* diastereoisomers on the silica column was obtained by using a mobile phase consisting of *n*-hexane:*tert*-butyl methyl ether (96:4) (v/v) at a flow rate of 1 ml min^{-1}. The *cis* and *trans* peaks were then switched to the enantioselective OJ column separately in two subsequent injections. The resolution of the four *trans* stereoisomers was accomplished by using *n*-hexane:*tert*-butyl methyl ether (90:10) (v/v), while the mobile phase composition for the four *cis* stereoisomers consisted of *n*-hexane:isopropanol (99.3:0.7) (v/v). The CD based detection system allowed the determination of the elution order on the basis of the CD signals of the single stereoisomers, together with the injection of pure stereoisomers. Under the final conditions, the validated method was applied to the determination of stereoisomeric composition and absolute configuration of the prevailing stereoisomers of real samples, i.e. commercial batches of different sources of *d*-allethrin.

Keywords: Enantiomer separation; Stereoisomeric composition; Absolute configuration; Allethrin

1. Introduction

Commercially synthetic pyrethroids account for more than 30% of the world trade in insecticides. The increasing consumption and the broad application of these insecticides require reliable analytical methods for the quality control of each batch of product. Several synthetic pyrethroids are marketed either as single most active isomer or as selected mixtures of stereoisomers, in order to enhance activity, minimising toxicity. Since pyrethroid stereoisomers can exhibit significantly different biological activity and toxicity [1–3], registration procedures and quality control assays require accurate specifications of the stereoisomeric composition of the synthetic product.

The *d*-allethrin (DA) is one of the most used commercial products. It is a mixture of esters (four stereoisomers) derived from (1*R*, *trans*)- and (1*R*, *cis*)-chrysanthemic acid (CA) in the 80/20 ratio and (*R*,*S*)-allethrolone (Fig. 1). Owing to the presence of three stereogenic centres in the molecule, allethrin presents eight stereoisomers and therefore commercial DA can contain the other four stereoisomers as impurities, arising from the (1*S*, *trans*)- and (1*S*, *cis*)-chrysanthemic acid and (*R*,*S*)-allethrolone.

It is urgent for industries involved in the synthesis and distribution of this insecticide to control the stereoisomeric

* Corresponding author. Tel.: +39 051 2099742; fax: +39 051 2099734.
E-mail address: vincenza.andrisano@unibo.it (V. Andrisano).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.06.075

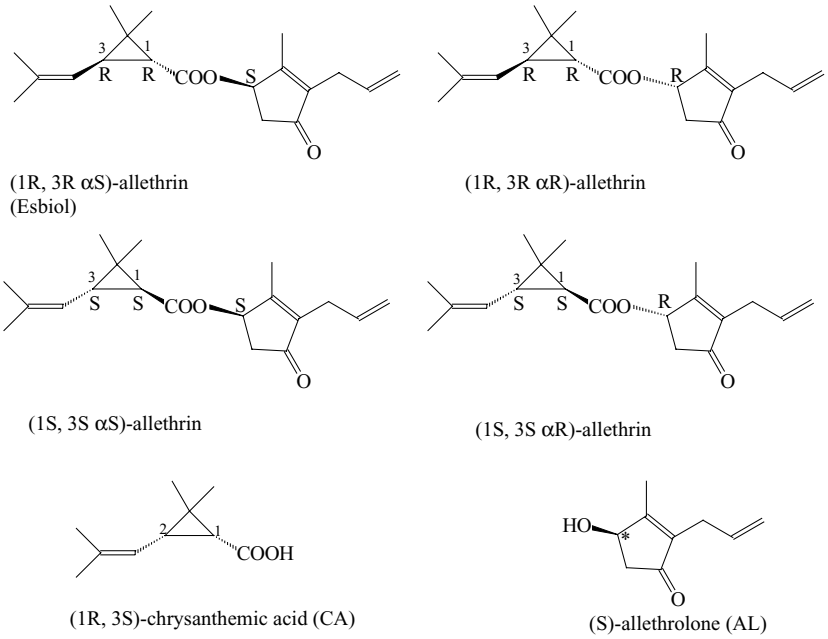

Fig. 1. Structures of *trans*-allethrin stereoisomers, (1*R*, 3*S*) *cis*-chrysanthemic acid (CA) and (*S*)-allethrolone.

composition of DA batches by a chromatographic method able to baseline resolve the possible eight stereoisomers in DA in order to determine the stereoisomeric composition and the absolute configuration of each stereoisomer.

Up to now only one paper reports the direct resolution of the eight stereoisomers of allethrin [4], using a stationary phase derived from (*S*) *tert*-leucine and (*S*)-1-(α-naphthyl)ethylamine chemically bonded to silica via a propyl spacer, but we could not satisfactorily reproduce it in our laboratory. Several papers attempted the allethrin stereoisomers resolution by using chiral GC and HPLC methods [5–13]. Liquid and gas chromatographic separations of the allethrin and cypermethrin were investigated with various achiral and chiral stationary phases. Diastereomeric and enantiomeric selectivity was observed for cypermethrin on a Pirkle-type chiral liquid chromatographic stationary phase, but very strong interactions and therefore long retention times prevented the separation of allethrin on this phase [10]. Only *trans*-allethrin isomers were separated on a chiral β-cyclodextrin RP-HPLC column [10]. The chiral separation of allethrin stereoisomers was partially achieved by direct GC analysis on substituted cyclodextrins and by enantioselective HPLC analysis on Pirkle column type 1-A; however, seven peaks were obtained because one of the two *cis* diastereoisomers was not resolved [5,13]. Hence, in these conditions, the enantiomeric composition of the eight stereoisomers of allethrin cannot be accurately determined.

Therefore, in order to successfully obtain the resolution of the eight DA stereoisomers, we developed a two-dimensional achiral/chiral HPLC method with circular dichroism (CD) detection.

The diastereomeric separation of *trans* and *cis* fractions of synthetic allethrin was first optimized and then the enantiomeric resolution and determination of the elution order of the eight stereoisomers was achieved using enantioselective conditions.

2. Experimental

2.1. Materials

rac-Allethrin (80/20 *trans*/*cis*) [2,2-dimethyl-3-(2-methyl-propenyl)-cyclopropanecarboxylic acid 3-allyl-2-methyl-4-oxo-cyclopent-2-enyl ester] and *d*-allethrin [mixture of esters derived from (1*R*, *trans*)- and (1*R*, *cis*)-chrysanthemic acid in the 80/20 ratio and (*R*,*S*) allethrolone] were obtained from Jiangsu Yangnong Co. Ltd. (China). (*S*)-4-Hydroxy-3-methyl-2-(2-propenyl)-2-cyclopenten-1-one [(*S*)-allethrolone ((*S*)-AL)] (e.e. 50%) was prepared from racemic allethrolone as described in US Patent 4,005,146. Patent (ASSIGNEE ROUSSEL-UCLAF); racemic allethrolone (*rac*-AL) was prepared as previously reported [14]. Both were characterized by HRGC/MS with a 5% phenyl 95% dimethylpolysiloxane column. The enantiomeric composition of the obtained allethrolone sample [75(*S*)–25(*R*)] was determined by GC/MS with a cyclodextrin enantioselective column (Restek Rt-βDEXsm, 30 m, 0.32 mm i.d., 0.25 μm film thickness). This stationary phase contains 2,3-di-*O*-methyl-6-*O*-*tert*-butyl dimethylsilyl β cyclodextrin doped into 14% cyanopropylphenyl/86% dimethyl polysiloxane as chiral selector and was supplied by Superchrom (Milano, Italy).

2,2-Dimethyl-3-(2- methyl- propenyl)-cyclopropanecarboxylic acid stereoisomers (chrysanthemic acid) were obtained by hydrolysis of correspondent methyl esters, commercially available from AgroChemie (Hungary). CA

samples were purified by distillation and their stereoisomeric composition determined by enantioselective GC.

Standard *d*-allethrin was purified by flash chromatography, identified by NMR and characterized by HRGC and HPLC.

Standard (1*R*, *trans* α *S*)-allethrin (Esbiol) and standard (1*R*, *trans* α *R*,*S*)-allethrin (Bioallethrin) were purchased from Jiangsu Yangnong Chemical Co. Ltd. (Yangzhou, Jiangsu, China).

The *cis*- and *trans*-allethrin diastereoisomers were obtained by chromatographic resolution of *rac*-allethrin upon a silica HPLC column (250 mm × 4.6 mm i.d.) (Alltech Italia, Milano Italy) with a mobile phase consisting of *n*-hexane:*tert*-butyl methyl ether (96:4) (v/v) at a flow rate of 1 ml min^{-1}. Low loading repetitive injections (30 μg each injection, one injection every 20 min) allowed the collection of about 1 mg of each *cis* and *trans* stereoisomeric fraction in 30 h. The purities of the single *cis* and *trans* fractions were determined by chromatographic analysis using the same method employed for the preparative resolution. All solvents used for the preparation of the solutions and the mobile phases were HPLC grade from Merck.

2.2. Instrumentation

A Jasco HPLC system was used, consisting of a Jasco PU-1585 solvent delivery system connected to a UV-1575 detector, detection at 230 nm, a Reodyne model 7725 injector with a 20 μl loop was used.

CD and UV chromatograms were simultaneously obtained using a Jasco PU-980 solvent delivery system, connected to the CD-995 chiral detection system, equipped with a micro (40 μl, 5 cm pathlength) HPLC flow cell, and a Jasco MD 910 multiwavelength detector.

CD spectra for (*S*)-AL (e.e. 50%) (2 mg/ml in methanol), chrysanthemic acid stereoisomers (2 mg/ml in methanol), and (1*R*, *trans* α *S*)-allethrin (esbiol) (0.1 mg/ml in methanol) were recorded on a Jasco J810 spectropolarimeter in the spectral range 185–400 nm using 1 mm and 0.1 mm pathlength cells at room temperature. Spectra were recorded at 0.5 nm intervals.

A silica monolithic HPLC column (100 mm × 4.6 mm i.d., Merck, Germany) was coupled orthogonally (Fig. 2) to a Chiralcel OJ (250 mm × 4.6 mm i.d.), Daicel Chemical Industries Ltd., column (250 mm × 4.6 mm i.d.) by means of a six port switching valve (Gilson "Valvemate" system, valve 7000, model 3299).

2.3. Chromatographic conditions

Preliminary achiral chromatographic separation of *trans* and *cis rac*-allethrin diastereoisomers was achieved by using a silica monolithic HPLC column with a mobile phase consisting of *n*-hexane:*tert*-butyl methyl ether (98:2) (v/v)

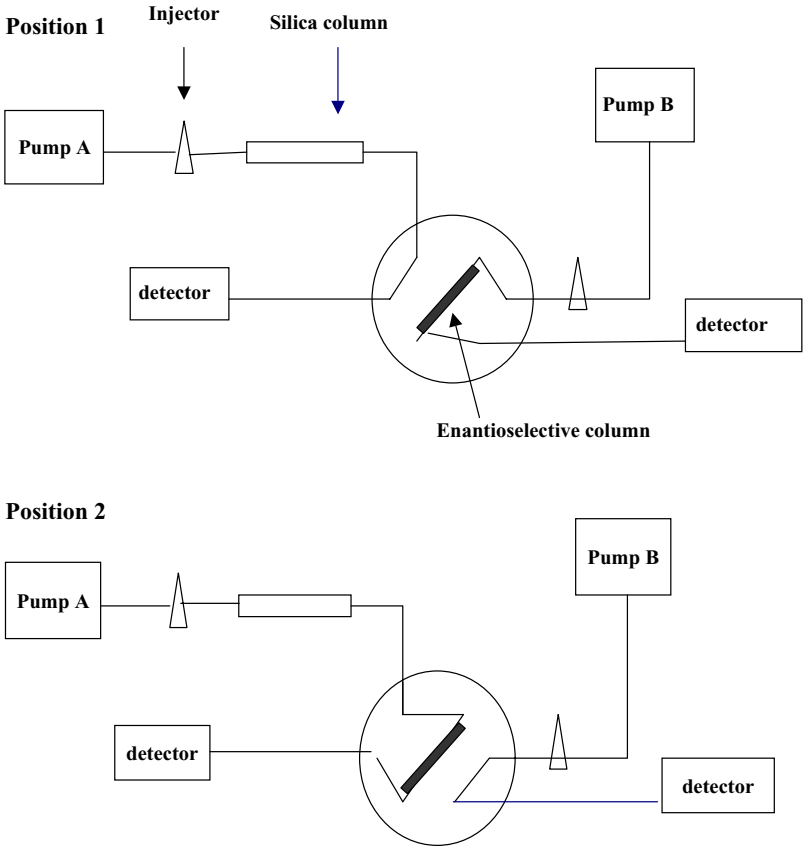

Fig. 2. Schematic representation of the two-dimensional achiral/chiral HPLC system.

(mobile phase A) at a flow rate of 1 ml min^{-1} with UV detection at 230 nm. The two individual fractions of *cis* and *trans* stereoisomers were then separately analysed by enantioselective HPLC.

The resolution of the four *trans* and the four *cis* stereoisomers was accomplished by using a Chiralcel OJ stationary phase. The mobile phase consisted of *n*-hexane:*tert*-butyl methyl ether (90:10) (v/v) at a flow rate of 1 ml min^{-1} for the four *trans* stereoisomers resolution (mobile phase B_2), while the mobile phase composition for the four *cis* stereoisomers consisted of *n*-hexane:isopropanol (99.3:0.7) (v/v) (mobile phase B_1), at a flow rate of 0.5 ml min^{-1}, with circular dichroism and UV detection at 320 nm.

In the orthogonal HPLC system the *cis* and *trans* stereoisomers were separated on the monolithic silica column with the mobile phase A at a flow rate of 1 ml min^{-1} with UV detection at 230 nm and were then switched to the stereoselective OJ column separately in two subsequent injections under the chromatographic conditions above described (B_1 and B_2) (Fig. 2).

2.4. Absolute configuration assignment

Absolute configuration assignment of allethrin stereoisomers separated by enantioselective HPLC was obtained by CD detection and involved, where possible, chromatography of individual standard isomers [i.e. (1*R*, *trans* α *S*)-allethrin] or enriched standard samples such as DA.

2.5. Stereoisomeric composition determination

Commercial samples of DA from various sources were dissolved in *n*-hexane (c = 0.2 mg/ml) and injected onto the achiral/chiral HPLC system. The percentage of *trans* (T%) and *cis* (C%) in the sample was calculated by using the peak areas obtained in the achiral separation.

The percentages of each stereoisomer in the DA sample (stereoisomeric composition [15]) were calculated by integrating the areas of the single stereoisomers of *trans* and *cis* DA and applying the following formula for each stereoisomer. As an example:

$$(1R,\ trans\,\alpha\,S)\text{-allethrin}\,(\%) = \left[\frac{A(1R, trans\,\alpha\,S)}{\sum A_{trans}}\right] \times \mathrm{T}\%$$

where A is the peak area of the selected *trans* isomer, $\sum A_{trans}$ the sum of all *trans* peak areas and T% is the *trans* isomers percentage determined in the achiral separation.

3. Results and discussion

3.1. Cis and trans allethrin diastereomeric separation

Besides chemical purity, which was object of a previous study [16], allethrin quality control has to deal with stereoisomeric composition determination.

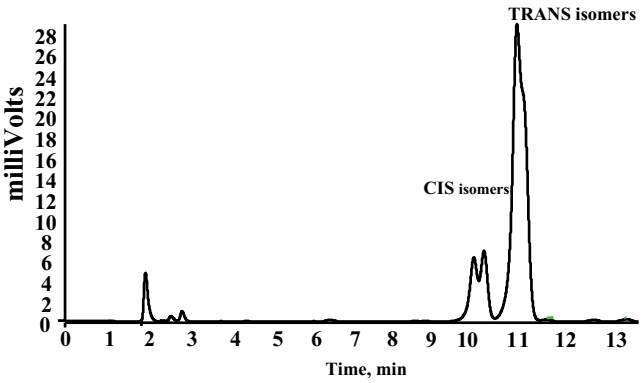

Fig. 3. HPLC separation of *rac*-allethrin (80/20 *trans*/*cis*). Chromatographic conditions: silica monolithic HPLC column (100 mm × 4.6 mm i.d., Merck, Germany) with a mobile phase consisting of *n*-hexane:methyl *tert*-butyl ether (98:2) (v/v) (mobile phase A) at a flow rate of 1 ml min^{-1} with UV detection at 230 nm.

Owing to the presence of three stereogenic centres in the molecule (Fig. 1), allethrin may present eight stereoisomers. In DA, four stereoisomers are represented by the two prevailing stereoisomers derived from (1*R*, *trans*)- and (1*R*, *cis*)-chrysanthemic acid esterified with *rac*-(AL): namely (1*R*, *trans* α *R*); (1*R*, *trans* α *S*) and (1*R*, *cis* α *R*); (1*R*, *cis* α *S*), while the other four arise from the two CA diastereomers (1*S*, *trans*) and (1*S*, *cis*), combined with *rac*-AL, present as impurities (Fig. 1).

Pyrethroid stereoisomers are often readily separated into diastereomers under normal phase HPLC conditions [10]. Although silica columns have normally been used for this purpose, the rapid equilibration of monolithic columns with the mobile phase and the high reproducibility of the separations, makes them suitable for time saving and reliable quality control [18]. Therefore, a monolithic silica column was chosen for the *cis* and *trans* allethrin diastereomer separation. Typical example of the separation for allethrin diastereomers is shown in Fig. 3, by using as mobile phase a mixture of *n*-hexane and methyl *tert*-butyl ether. This organic modifier resulted as a high selectivity solvent for this application and for the subsequent chiral separation. Under the described chromatographic conditions, baseline resolution between the *cis* and *trans* diastereomers was readily obtained, the *cis* pairs being eluted before the *trans*. Concerning the *cis*-isomers chromatographic behaviour, it should be noted the splitting of the resulting peak due to the partial separation of the two diastereomers of the four *cis*-stereoisomers. The elution order was attributed by injecting standard samples with defined stereochemistry, in particular, a sample with a high *trans* content (DA 80/20 *trans*/*cis*).

Under the same chromatographic conditions, preparative chromatography of *rac*-allethrin and DA was performed and the two *cis* and *trans* diastereomeric fractions were separately collected in mg scale and their purity checked with the same chromatographic system.

Table 1
Cis and *trans* allethrin stereoisomers chromatographic parameters on chiral OJ column

	(1*S*, *cis* α *S*)	(1*R*, *cis* α *S*)	(1*R*, *cis* α *R*)	(1*S*, *cis* α *R*)	(1*S*, *trans* α *R*)	(1*S*, *trans* α *S*)	(1*R*, *trans* α *R*)	(1*R*, *trans* α *S*)
k	9.9	12.2	14.3	24.6	3.7	4.8	7.3	11.0
α[a]		1.2	1.2	1.7		1.3	1.5	1.5

[a]Selectivity factor determined by the ratio between *k* of two adjacent peaks.

3.2. Chiral separation

The next step consisted of the enantioselective HPLC analysis of each diastereomeric *cis* and *trans* fractions for stereoisomer resolution. The same stationary phase (Chiralcel OJ) resulted successful for both fractions: each stereoisomer was baseline resolved by using two different types of mobile phases (Fig. 4). This stationary phase was selected because contains cellulose esters [cellulose tris (4-methylbenzoate)]. We thought this chemical structure would have had a certain degree of similarity with the analytes better than the cellulose carbamates. Aprotic modifiers such as acetonitrile, ethyl acetate, methylene chloride, etc. were reported to have a reduced tendency to interact by hydrogen bonding with the modified cellulose stationary phase, providing higher enantiomeric resolution for 'ester' compounds that also compete for these hydrogen-bonding, chirally-active sites [17]. On these premises, various aprotic organic modifiers were tested to improve chiral resolution and methyl-*tert*-butyl ether, used also as polar modifier in the previous diastereomeric separation with the silica column, resulted particularly selective for the *trans* stereoisomers separations. In this case, the mobile phase consisted of *n*-hexane:methyl-*tert*-butyl ether (90:10) (v/v), while the mobile phase composition for the four *cis* stereoisomers consisted of *n*-hexane:isopropanol (99.3:0.7) (v/v) (Fig. 4a and b), because only three peaks were obtained by using the mobile phase adopted for the *trans* stereoisomers separation. In Table 1, the capacity factor values [$k = (t - t_0/t_0)$] for each stereoisomers and enantioselectivity factors ($\alpha = k'_2/k'_1$) determined between two adjacent peaks are reported.

3.3. Absolute configuration attribution

In order to attribute the absolute configuration to each eluted stereoisomer, CD spectra of selected standards were acquired (Fig. 5). In particular, the comparison of the CD spectrum profile of (1*R*, *trans* α *S*)-allethrin (esbiol) with the (*S*)-AL and CA spectra, revealed CD bands selectively related to the alcohol moiety and to the acid. In fact, the CD band with λ_{max} at 320 nm is associated to the transition n–π* of alfa–beta unsaturated carbonyl chromophore of the

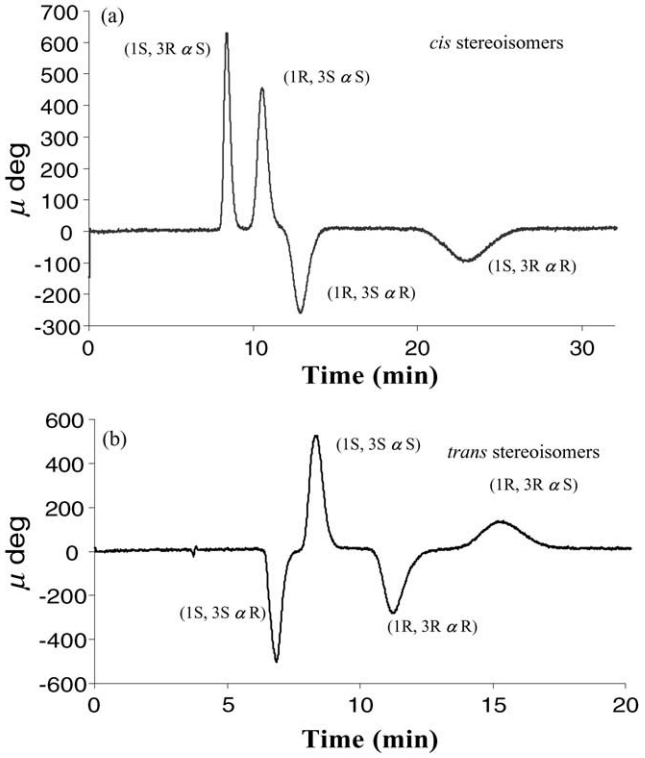

Fig. 4. Chiral HPLC analysis with OJ column and CD detection at 320 nm of (a) *cis* fraction of *rac*-allethrin stereoisomers and (b) *trans* fraction of *rac*-allethrin stereoisomers. Chromatographic conditions: as described in Section 2.

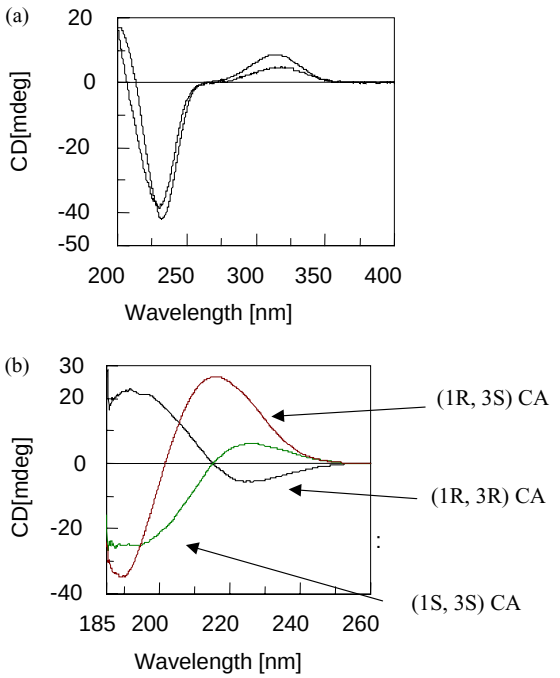

Fig. 5. (a) Overlaid CD spectra of (*S*)-allethrolone (AL) (—) and (1*R*, *trans* α *S*)-allethrin (esbiol) (- - -) and (b) CD spectra of chrysanthemic acid (CA) stereoisomers.

alcohol moiety, whereas the band at higher energy (λ_{max} at 230 nm) is also shown in the CD spectra of CA stereoisomers; in particular, λ_{max} at 230 nm was found for (1*R*, *trans*)-CA and λ_{max} at 218 nm for (1*R*, *cis*)-CA.

On the basis of CD spectra analysis, selective chromatograms were then obtained by the choice of the wavelength for the CD monitoring. By fixing the CD detection at 320 nm, the attribution of the absolute configuration at AL stereogenic centre of allethrin stereoisomers was accomplished by the sign of the chromatographic peaks. Negative peaks were indicative of (*R*)-configuration at the alcohol moiety and positive peaks were related to (*S*)-configuration (Fig. 4). These attributions were then confirmed by injecting standard stereoisomers [(1*R*, *trans* α *S*)-allethrin (esbiol) and its racemic mixture (bioallethrin)] and pure *cis* stereoisomers (1*R*, *cis* α *R*,*S*)-allethrin.

The next step of the analysis was addressed to the C1,C3 stereochemistry attribution for each individual peak. With regard to *trans* stereoisomers, the last eluting peak in the related chromatogram (Fig. 4b) showed the same *k* and CD signal as esbiol and was therefore identified as (1*R*, *trans* α *S*)-allethrin. The third eluting peak was characterized as having the same C1,C3 absolute configuration but the opposite, (α *R*) alcohol absolute configuration, by injecting (1*R*, *trans* α *R*,*S*)-allethrin (bioallethrin).

The C1,C3 absolute configuration of the first two eluting peaks was consequently deduced as being (1*S*, *trans*) by the previous outcomes and the alcohol moiety stereocenter configuration assessed by the sign of the CD detection system at 320 nm.

In relation to *cis* stereoisomers, as well as for the *trans* fraction, the alcohol chiral carbon was characterized by the CD sign (Fig. 4a). The C1,C3 stereochemistry was attributed on the basis of the chromatographic behaviour of standard (1*R*, *cis*)-DA stereoisomers.

3.4. Automated analysis by column switching

Coupled chiral/achiral HPLC system were described to separate the enantiomers of various drugs first and then trapping and compressing them in a variety of hydrophobic phases [19,20]. In this work, we developed an achiral/chiral HPLC system to obtain the *cis* and *trans* diastereomeric separation first and then the chiral separation of allethrin stereoisomers. In order to automate the system and obtain the on-line achiral/chiral DA stereoisomers HPLC separation, a two-dimensional chromatographic system was assembled, by means of a six port switching valve (Fig. 2). For this purpose an additional pump and a switching valve are required. The allethrin sample is injected into the monolithic silica column, which is conditioned by mobile phase A, with the switching valve in position 1. Simultaneously, the chiral column is conditioned with the specific mobile phase B (either B_1 for *cis* or B_2 for *trans* stereoisomers separation). A first sample injection with the switching valve in position 1 is performed to define the times for the switching steps. In a following injection, at t_1, corresponding to the starting *cis* elution peak, the valve is switched to position 2 and maintained in this position until t_2, time when the *cis* peaks are completely eluted. In this condition the two columns are connected together and the *cis* fraction is completely eluted with the mobile phase A and transferred to the enantioselective column. Afterwards, the valve is switched to its initial position 1 and the *trans* fraction goes to waste while *cis* stereoisomers are separated onto the chiral column with the specific mobile phase B_1, and directed to the detector UV (Fig. 6a). Finally, the switching valve in position 1 allows the system to be ready for the next sample injection. All *cis* DA samples can be processed in this way and then *trans* fractions can be analysed by subsequent (following) sample injections of the previously processed solutions. In the *trans* enantioselective analysis, OJ column is conditioned with mobile phase B_2, specific for *trans* stereoisomers, and the valve is switched to position 2 at t_3, which corresponds to the starting *trans* peak elution time. At t_4, end of *trans* peak, the valve is switched to position 1 and the *trans* stereoisomers are separated on the chiral column and directed to the UV detector (Fig. 6b). The recovery of the *cis* and *trans* fractions from the silica column was verified by individually injecting the pure racemic *cis* and *trans* fractions and verifying that the area of each obtained enan-

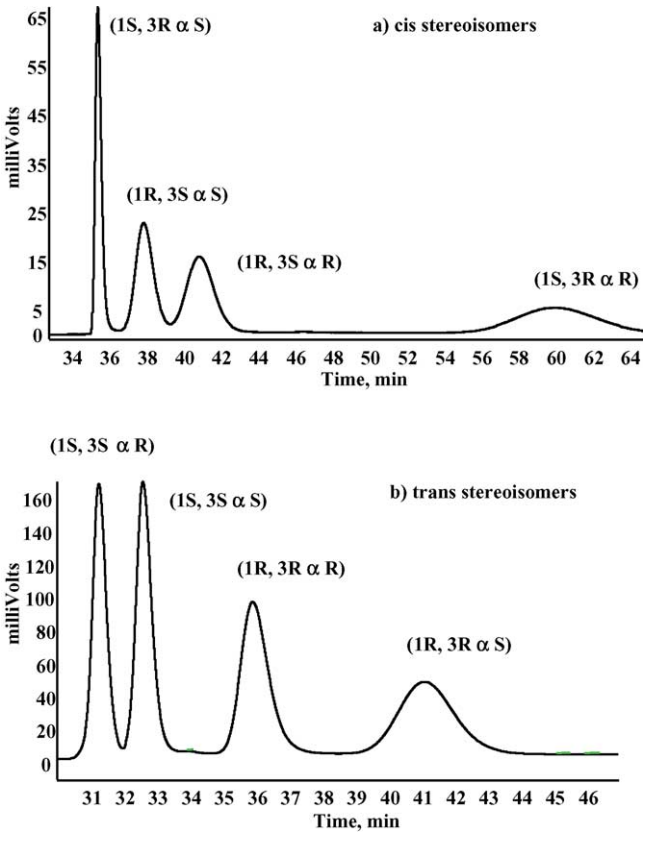

Fig. 6. Two-dimensional achiral/chiral HPLC analysis of *rac*-allethrin (a) chromatogram of the *cis* fraction and (b) chromatogram of the *trans* fraction, switched to the enantioselective column (OJ), UV detector, λ = 230 nm.

Table 2
Stereoisomeric DA samples composition (%)

(1*S*, *cis* α *S*)	(1*R*, *cis* α *S*)	(1*R*, *cis* α *R*)	(1*S*, *cis* α *R*)	(1*S*, *trans* α *R*)	(1*S*,*trans* α *S*)	(1*R*, *trans* α *R*)	(1*R*, *trans* α *S*)
0.43 ± 0.08	9.48 ± 0.13	9.67 ± 0.11	0.35 ± 0.02	1.52 ± 0.31	1.70 ± 0.22	38.60 ± 0.29	38.27 ± 0.05
0.35 ± 0.07	10.78 ± 0.17	9.44 ± 0.14	0.29 ± 0.04	1.11 ± 0.30	1.28 ± 0.30	40.36 ± 0.23	36.36 ± 0.22

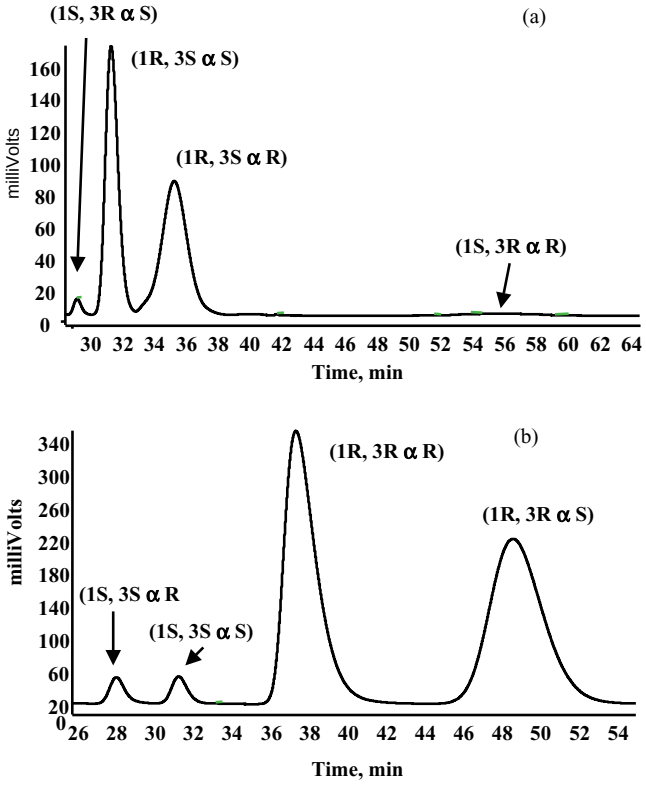

Fig. 7. Two-dimensional achiral/chiral HPLC analysis of a DA sample (a) chromatogram of the *cis* fraction and (b) chromatogram of the *trans* fraction, switched to the enantioselective column (OJ), UV detector, λ = 230 nm.

tiomeric peak had the same value (Fig. 6a and b). The limit of detection (LOD) for each enantiomer increases by increasing the retention time and ranges from 0.03% to 0.1%.

By using this achiral/chiral chromatographic system, commercial samples of DA were analysed. In Fig. 7, typical chromatograms of *trans* and *cis* DA column switching analysis are reported. The results of two DA batches are reported in Table 2. The results obtained were found in agreement with the declared content for the prevailing stereoisomers.

4. Conclusions

The reported two-dimensional achiral/chiral HPLC method resulted useful for the baseline chiral separation of all the eight stereoisomers of allethrin and for the enantiomeric composition and purity determination and therefore for the quality control of DA from different commercial sources. The proposed method offers the advantageous opportunity of the automation in order to process a large number of batches. The CD based detection system resulted suitable for a selective analysis.

Acknowledgements

This research was supported by a grant from MIUR (project no. 066716, Rome, Italy) and a grant from Endura S.p.A. The authors thank Professor P. Biscarini (Facoltà di Chimica Industriale, Università di Bologna, Italy), for the availability of the J-810 Jasco spectropolarimeter.

References

[1] R. Hirata, Quim. Nova 18 (1995) 368.
[2] N. George, M. Kalyanasundaram, J. Sci. Ind. Res. 53 (1994) 933.
[3] N. Kurihara, J. Miyamoto, G.D. Paulson, B. Zeeh, M.W. Skidmore, R.M. Hollingworth, H.A. Kuiper, Pestic. Sci. 55 (1999) 219.
[4] N. Oi, H. Kitahara, K. Reiko, J. Chromatogr. 515 (1990) 441.
[5] I.H. Hardt, C. Wolf, B. Gehrcke, D.H. Hochmuth, B. Pfaffenberger, H. Huehnerfuss, W.A. Koenig, J. High Resolut. Chromatogr. 17 (1994) 859.
[6] N. Oi, H. Kitahara, Y. Matsushita, N. Kisu, J. Chromatogr. A 722 (1996) 229.
[7] R.S. Burden, A.H.B. Deas, T. Clark, J. Chromatogr. 391 (1987) 273.
[8] N. Oi, H. Kitahara, T. Doi, J. Chromatogr. 254 (1983) 282.
[9] N. Oi, T. Doi, H. Kitahara, Y. Inda, Bunseki Kagaku 30 (1981) 552.
[10] J.P. Kutter, T.J. Class, Chromatographia 33 (1992) 103.
[11] J. Zhou, J. Liu, M. Wang, S. Jiang, Fenxi Huaxue 30 (2002) 504.
[12] G.R. Cayley, B.W. Simpson, J. Chromatogr. 356 (1986) 123.
[13] G. Lisseter, S.G. Hambling, J. Chromatogr. 539 (1991) 207.
[14] M.S. Schechter, N. Green, F.B. LaForge, J. Am. Chem. Soc. 71 (1949) 3165.
[15] V. Schurig, Enantiomer 1 (1996) 139.
[16] M. Bragieri, A. Liverani, M.C. Zanotti, V. Borzatta, J. Fiori, V. Cavrini, V. Andrisano, J. Sep. Sci. 27 (2004) 89.
[17] K.M. Kirkland, J. Chromatogr. A 718 (1995) 9.
[18] A.M. van Nederkassel, A. Aerts, J. Pharm. Biomed. Anal. 32 (2003) 233.
[19] L. Silan, P. Jadaud, L.R. Whitfield, I.W. Wainer, J. Chromatogr. 532 (1990) 227.
[20] A. Walhagen, L.E. Edholm, J. Chromatogr. 473 (1989) 371.