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Short Communication

Chiral high-performance liquid chromatography of synthetic pyrethroid insecticides

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ABSTRACT

Fifteen synthetic pyrethroid insecticides were examined by HPLC using a Pirkle column. Complete separation of enantiomer peaks was obtained in almost all cases. Some formulated products were also examined and comments are made about the suitability of the technique for routine analysis.

INTRODUCTION

It is well known that different enantiomers of pesticides and pharmaceuticals can have significantly different biological activity. It therefore seems increasingly likely that national registration procedures will require specification of the enantiomeric content of formulated products [1]. This implies the need for valid analytical methods for enantiomer determination. High-performance liquid chromatography (HPLC) using chiral columns is currently the foremost technique for this purpose though chiral detection, derivatisation with chiral reagents and chiral gas–liquid chromatography all have a role to play.

Cayley and Simpson [2] reported that optical isomers of a variety of synthetic pyrethroid insecticides could be separated by normal-phase HPLC using Pirkle (type 1-A ionic or covalent) columns and the contribution of other workers to this field has been reviewed [3,4]. However, only standard solutions of the pyrethroids have been examined. In the present work the results of Cayley and Simpson have been confirmed, and the range of compounds studied has been extended. Formulated products have also been examined to check the robustness and usefulness of chiral HPLC for these.

EXPERIMENTAL

Pirkle columns (250 × 4.6 mm I.D.) both ionic type 1-A and covalently bonded were obtained from Technicol (Stockport, U.K.). A short guard column of 5-μm

Spherisorb NH_2 (Phase Separations, Deeside, U.K.) was employed. Manual injection was used with a Rheodyne injector, a Pye Unicam PU4010 pump and PU4020 detector set at 230 nm.

Samples of technical pyrethroids and formulated products were obtained as gifts from the manufacturers. Solutions were prepared with about 10 mg of active ingredient in 10–100 ml hexane.

RESULTS

Optimum separation of enantiomers requires careful adjustment of chromatographic parameters. Our preferred experimental conditions and results are summarised in Table I.

Complete separation of the four enantiomers of *d*-allethrin was achieved readily. The racemic *dl*-allethrin could not be fully separated and our findings are in line with earlier work [2]. α-Cypermethrin contains chiefly the two most bioactive *cis* enantiomers of cypermethrin, which are easily separated from each other. This compound is quite a good check-compound to monitor column performance as the colourless crystalline technical material is easy to handle. It was possible to obtain eight peaks from cypermethrin (Fig. 1a) representing an improvement on studies reported in the literature, but the separation was unreliable, and could only be repeated in two consecutive injections before collapsing to seven peaks (Fig. 1b). This may be due to the nature or number of chiral binding sites on the column changing with time. Cyhalothrin and λ-cyhalothrin posed no difficulties yielding four and two peaks respectively. Cyfluthrin yielded eight discernible peaks but overlap of two enantiomer

TABLE I

HPLC SEPARATION OF PYRETHROIDS ON PIRKLE COLUMNS

Compound	Column[a]	Theoretical No. of peaks	No. of peaks observed	Mobile phase[b]	Flow-rate (ml/min)
dl-Allethrin	I	8	7	0.15	0.8
d-Allethrin	I	4	4	0.15	1.5
Cyfluthrin	C	8	8	0.05	1.0
λ-Cyhalothrin	C	2	2	0.15	1.0
Cyhalothrin	C	4	4	0.15	1.0
α-Cypermethrin	C,I	2	2	0.15	1.3
Cypermethrin	I	8	8	0.15	1.3
Fenpropathrin	I	2	2	0.15	0.8
Fenvalerate	C,I	4	4	0.15	2.0
Flucythrinate	C	2	2	0.05	2.0
Flumethrin	C	4	4	0.05	2.0
Permethrin	I	4	4	0.05	0.8
d-Phenothrin	I	2	2	0.05	0.8
Resmethrin	I	4	4	0.05	0.8
Tetramethrin	I	4	4	0.15	2.5

[a] I = Ionic column; C = covalent column. Where both types were used the conditions given are for the ionic column.

[b] Mobile phase is expressed as the percentage of propan-2-ol in hexane.

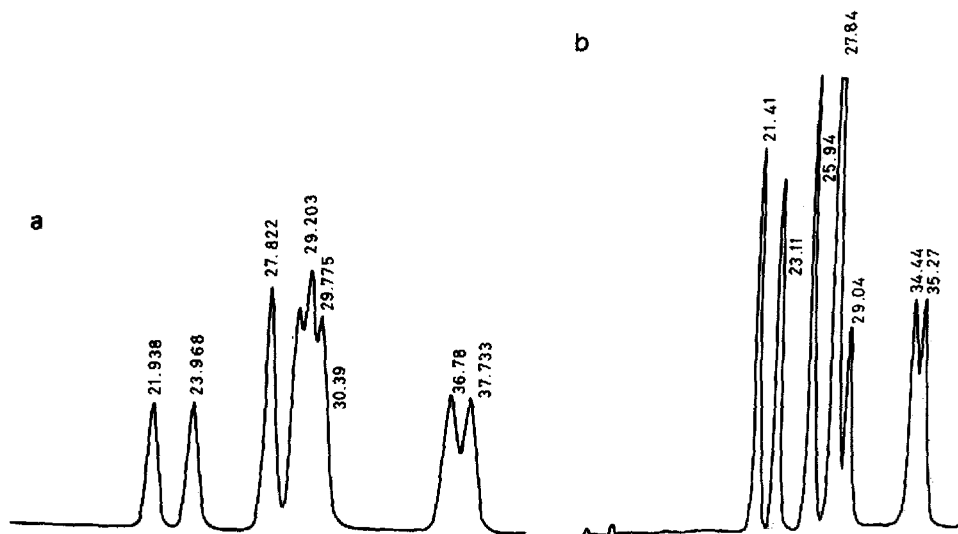

Fig. 1. Chiral separation of cypermethrin enantiomers. (a) 8 peaks; (b) 7 peaks. Pirkle ionic column with Spherisorb NH_2 guard column. Mobile phase: hexane containing 0.15% propan-2-ol, flow-rate 1.3 ml/min. Numbers at peaks indicate retention times in min.

peaks could not be entirely resolved despite long retention times (Fig. 2). Flucythrinate contains two chiral centres but is defined as possessing *S*-stereochemistry in the acid moiety. Two peaks were thus observed. Four peaks were obtained in the analysis of flumethrin, a pyrethroid used in veterinary practice, which contains three chiral centres. It was considered that the sample examined contained only negligible amounts of the four *cis*-isomers obtained. Separation of tetramethrin yielded the theoretical number of peaks.

Formulations of cypermethrin, fenvalerate and permethrin, including wettable powders and emulsifiable concentrates gave similar results to the technical samples,

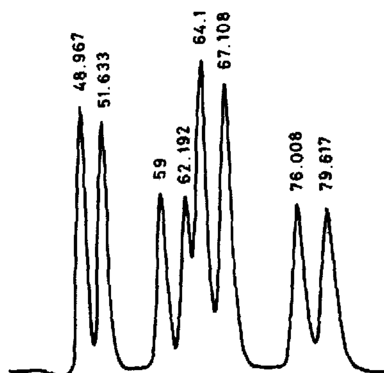

Fig. 2. Chiral separation of cyfluthrin. Pirkle covalent column with Spherisorb NH_2 guard column. Mobile phase: hexane containing 0.05% propan-2-ol, flow-rate 1.0 ml/min. Numbers at peaks indicate retention times in min.

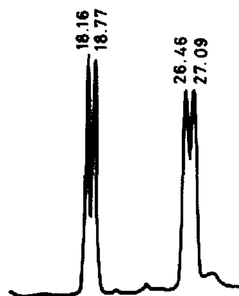

Fig. 3. Separation of enantiomers of formulated permethrin. Pirkle ionic column with Spherisorb NH_2 guard column. Mobile phase: hexane containing 0.05% propan-2-ol, flow-rate 0.8 ml/min. Numbers at peaks indicate retention times in min.

with identical numbers of separated peaks, though the baselines showed more background noise (Fig. 3). In the case of one permethrin formulation, coelution of the synergist piperonyl butoxide with one of the active ingredient enantiomer peaks hindered quantitation.

It was noticed that the commercial ionic column rapidly lost selectivity when exposed to formulations, and required extensive cleaning using hexane–tetrahydrofuran (1:1) and rejuvenation using "Chiral Column Regenerating Solution" (J. T. Baker, Phillipsburg, NJ, U.S.A.) after two weeks of daytime use. It is not known which components of the formulations were responsible for the selectivity loss.

CONCLUSION

Chiral HPLC using Pirkle columns has been shown to effect the separation of a large range of synthetic pyrethroid technical materials and a few formulated products. The columns used may be insufficiently stable for regular analysis of formulated products.

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REFERENCES

1 E. J. Ariens, *Trends Pharm. Sci.*, 9 (1988) 317–318.
2 G. R. Cayley and B. W. Simpson, *J. Chromatogr.*, 356 (1986) 123–134.
3 E. Papadopoulou-Mourkidou, in J. Sherma (Editor), *Analytical Methods for Pesticides and Plant Growth Regulators, XVI, Specific Applications*, Academic Press, London 1988, pp. 196–206.
4 P. G. Baker, in R. Greenhalgh and T. R. Roberts (Editors), *Pesticide Science and Biotechnology: Report of the IUPAC Conference, Ottawa, 1986*, Blackwell Scientific, Oxford, 1987, pp. 329–332.